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A Probabilistic Model of Flower Fertility and Factors Influencing Seed Production in Winter Oilseed rape (*Brassica napus L.*)

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Abstract

The number of pods per plant and the number of seeds per pod are the most variable yield components in winter oilseed rape (WOSR). The production of a seed is the combination of several physiological processes, namely formation of ovules and pollen grains, fertilization of the ovules and development of young embryos, any problem in these processes may result in seed abortion or pod abortion. Both the number of ovules per pod and the potential for the ovule to develop into a mature seed may depend on pod position in the plant architecture and time of appearance. Furthermore, the expansion (basipetal) of ramifications is in inverse order of the initiation (acropetal) in WOSR. The complex developmental pattern of WOSR makes it difficult to analyze.
In this thesis, we first investigated the variability of the following yield components (a) ovules/pod, (b) seeds/pod, and (c) pods/axis in relation to two explanatory variables. These two variables include (1) flower and inflorescence position and (2) time of pod appearance, linked to the effect of assimilate availability. Based on these experiments, we developed a probabilistic model to simulate the number of ovules per ovary and seeds per pod according to the biological phenomena of flower fertility. The model can deduce the distribution of the number of pollen grains per stigma and distinguish the factors that influence the yield. Meanwhile, we compared our model to another model of flower fertility in kiwifruit developed by Lescourret et al., and improved the computation for the distribution of pollen grain number in our model.. In the last, we tested our model on the other species including cacao tree and soybean. The main contents are as follows:

1 The number and position of flowers and pods were recorded for the main stem (R0) and inflorescences R1, R4, R7, R9 and R11. The variety was Mendel. The results indicated that for the main stem, the number of ovules per pod decreased for a few ranks and then tended to increase and again to decrease at the end. On the ramifications R1 and R4, the number of ovules increased at first then remained constant with the pod rank, but it remained constant along the inflorescence on the other ramifications. However, the mean number of ovules per pod increased with ramifications from top to bottom. The number of seeds per pod did not vary with the pod rank at the basal positions and decreased afterwards along the inflorescence, but it did not differ between inflorescences. The number of ovules and seeds per pod did not vary with the time of pod appearance for the pods. However, the number of ovules and seeds per pod can be impacted by the time of pod appearance on the plant scale.

2 To analyze the effect of available assimilates on the yield components, different trophic states were created by clipping the main stem, ramifications or basal flowers. The results indicated that clipping the main stem or ramifications increased the number of ovules per pod, seeds per pod and pods per axis. However, clipping

all the ramifications and basal flowers only increased the number of pods on the main stem. In addition, clipping treatments increased the mean seed weight. Accordingly, we can conclude that assimilate availability is one of factors influencing the seed production.

3 The model of flower fertility was developed by combining different probability distributions. The model can simulate the number of viable ovules per ovary, the number of pollen grains per flower, the probability of seed viability and the survival probability of pod. Model parameters were estimated using a Generalized Least Square method with two years, clipping treatments, pod ranks, ramification ranks and four varieties. The results indicated that ovule viability, the intensity of pollination, assimilate availability and architectural effects can influence the seed production.

4 Two resampling methods were used to analyze the stability of the model. The coefficients of variation (CV) using jackknifing were smaller than that using bootstrapping. However, the results of the two methods indicated that the parameters in the model were quite stable. The CVs of the parameters were small except the variance of the number of ovules per pod. The CV was a little large with 3.3 for jackknife and 11 for bootstrap, respectively.

5 The estimation for the distribution of pollen grain number was improved by comparing our model to the model of flower fertility in kiwifruit developed by Lescourret et al. The flower fertility of model in kiwifruit computed the number of fertilized ovules using stochastic method. The results were good but it was time-consuming. The model developed in the thesis assumed that the ratio of ovule and pollen was 1:1. The smaller value of them was taken as the number of fertilized ovules. However, the studies in other species found that one pollen grain might not be enough to fertilize one ovule. Thus, we introduced one parameter k to estimate the proportion of effective pollen grains in the model, in turns, to compute the distribution of pollen grain number. The results indicated that the model can simulate the flower fertility in WOSR very well.

6 The model of flower fertility was used to simulate the number of seeds per pod in soybean and cacao tree. The results in soybean were good. The number of ovules and seeds per pod can be well calibrated. For the cacao tree, the model can estimate the number of ovules and seeds per pod with good pollination. However, the estimations were not good for the situation of poor pollination. We do not figure out why the results were not good.

Based on the field experiments and model estimations, the following conclusions can be drawn. The amount of available assimilates was the primary determinant of pod and seed production during the flowering period. Furthermore, the ovule viability and

pollination limitation could result in the decrease of the number of pods and the number of seeds per pod at the distal position of inflorescence. In addition, the distribution of resources was significantly affected by both the positions of pods within an inflorescence and the position of inflorescences within a plant in WOSR. The model of flower fertility could be a useful tool to study how to improve seed yield in flowering plants and the model can be applied to the other flowering plants.

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Chapter 1

Introduction

1.1 Context

Seed yield of oilseed rape is determined by several variables, including plant density, number of pods per plant, number of seeds per pod and individual seed weight [Diepenbrock, 2000]. Seed yield was significantly and positive correlated with the number of pod per plant and 1000-seed weight [Ozer et al., 1999]. Furthermore, Tuncturk et al. [Tuncturk and Ciftci, 2007] investigated the relationships between yield and some yield components of 16 oilseed rape cultivars by using correlation and path coefficient analysis. The results revealed that number of branches per plant, the number of pods per plant, 1000-seed weight and number of seeds per pod have shown a considerable direct positive effect on seed yield. Large variations exist in the yield components of oilseed rape among varieties [Ali et al., 2003] and between plants of the same variety grown in the same field [Malagoli et al., 2004].

Flowers provide the most trustworthy external characteristics for establishing relationships among angiosperm species. Flowers show remarkable variation in form and elaboration between angiosperm species and between plants in the same species. The pattern most commonly found is a reduction in the number or size of reproductive structures from proximal/early to distal/late flowers within the inflorescence [Buide, 2004; Lee, 1988], or a similar reduction in fruit set and/or seed set [Guitián and Navarro, 1996; Solomon, 1988]. In most flowering plants, the number of seeds is affected by low ratios of fruit-to-flower [Stephenson, 1981] and seed-to-ovule [Arathi et al., 1999]. In fact, only part of the flowers and ovules that are initiated form fruits and seeds [Bawa and Webb, 1984; Lloyd, 1980]. Abortions occur at several developmental stages, even in mature fruits [Arathi et al., 1999]. In addition, in some plants, a fruit with too few seeds may abort [Bertin, 1982; Ganeshaiah et al., 1986]. Particularly for plants with inflorescences, as their flower and fruit formation occur during a long period, and it is subjected to a great variability in various environmental conditions, the size and number of reproductive structures and the components of female reproductive success show marked variations among flowers within the inflorescence [Lee, 1988; Stephenson, 1980].

Numerous studies have investigated the relationships between reproductive effort and spatial position (proximal or distal), and time of opening (early or late) within the inflorescence. The probability of fruit set and number of seeds per fruit are often lower for distal/late-opening flowers than that for proximal/early-opening flowers [Guitián and Navarro, 1996]. The earliest opening flowers on an inflorescence are more likely to set fruit and produce more seeds than later opening flowers [Medrano et al., 2000]. The flowers located at a lower position of the inflorescence and which opened earlier showed higher fruit set than those at a higher position and which opened later within the inflorescences [Hiei and Ohara, 2002]. Likewise, fruit size [Wolfe, 1992], flower size and ovary size [Ashman, 1992], stamen number [Diggle, 1995], ovule number and seed number [Brookes et al., 2010; Burd et al., 2009; Cruden, 1977] and pollen production [Brunet and Charlesworth, 1995; Burd, 1995] have been observed to decline in a proximal to distal pattern.
Taken together, the flowers play an important role on reproductive success of flowering plants, which brings up a research subject that which factors impact the reproductive success and how they work.

1.2 Problematics

1.2.1 Causes of reproductive failure in flowering plants

An upper limit to the number of fruits that can be produced by an individual during a reproductive episode is set by the number of female flowers, while an upper limit to the number of seeds is set by the number of ovules within these flowers [Stephenson, 1981]. The fraction of this reproductive potential depends upon the number of pollinated flowers [Stephenson, 1981], the number of fertilized ovules [Bouttier and Morgan, 1992b], fruit/seed predation [Janzen, 1971], and the ability of the maternal parent to provide the necessary resources for development [Elizabeth, 1991]. Various non-exclusive factors have been put forward to explain variation of reproductive failure in plant species, such as resource competition [Arathi et al., 1999; Lee, 1988; Stephenson, 1980], non-uniform pollination [Berjano et al., 2006; Gruber and Claupein, 2007] and architectural effects [Diggle, 1995; Medrano et al., 2000; Vallius, 2000]. In this section, based on the previous studies, we can conclude about the factors influencing seed production and detail them in the following.

Ovule viability

Seed number per pod is determined by the number of ovules per ovary, the number of ovules fertilized and the number of fertilized ovules developing into seeds. The number of ovules per ovary is a genetic factor of plant and varies with genotype. The probability of ovule viability is quite stable for the same species [De Reffye, 1974]. Ovule viability

is described as the percentage of ovules with complete embryo sacs at flower opening [Bouttier and Morgan, 1992b]. Generally speaking, 30% of the ovules are sterile due to the absence of an embryo sac [Bouttier and Morgan, 1992b]. Within the terminal raceme, decreased ovule viability due to the sterility of ovules is one of the causes for the lower number of seeds per pod in the apical region compared to the basal region. If the proportion of ovules with embryo sac decreases according to the rank, pollination will be incomplete even if the amount of pollen grains is large, because not all the ovules of a pod will be fertilized [Charlesworth, 1989]. Thus, ovule viability is responsible for the lower number of seeds of late flowers.

Pollination limitation

Pollination limitation could also lead to a variation in pods and seeds [Brookes et al., 2010; Brunet and Charlesworth, 1995; Campbell and Halama, 1993; Harder and Aizen, 2010]. The magnitude of pollen limitation varies among flowers within an inflorescence, among inflorescences within a plant, and among plants within a season [Knight et al., 2005]. The failure of seed production may be caused by either reduced pollen production or poor pollen quality [Berjano et al., 2006]. Variations in reproductive traits and female reproductive success may also be attributable to differences in the quantity and quality of pollen [Burd, 1994]. On a given inflorescence or individual, the fruits from the first pollinated flowers are more likely to mature than those from flowers pollinated later [Lee, 1980; Stephenson, 1980]. Many, but not all, of the species with this pattern of flower and fruit abortion have inflorescences that develop acropetally (from top to bottom) [Stephenson, 1981]. The intensity and the efficiency of pollination are of crucial importance for the number of seeds [Brookfield et al., 1996; Wertheim, 1991]. Burd [Burd, 1994] reported that 62% of 258 species of angiosperms show evidence of pollen limitation. In addition, flower and seed abortion varies with flower position and flowering time [Hiei and Ohara, 2002]. Furthermore, flowering time is linked to the variation in pollen receipt and the abundance of pollinators [Medrano et al., 2000].
The quantity of pollen depends on the season, plot and cultivars. These differences in terms of pollination among cultivars observed in the same plot remain unexplained and may a specific attractiveness of cacao for insects and pollinators [De Reffye, 1974; Mossu et al., 1981]. Natural variation in the number of pollen grains deposited on stigmas also leads to variance in seed number among the fruits on a given individual. When this occurs, the fruits with a low seed number are often the most likely to abort [Bertin, 1982; Lee, 1980]. For example, Bertin found that 80% of fruits aborted if they received 200-800 pollen grains, whereas the flowers produced mature fruits if they received more than 800 pollen grains in *Campsis radicans* [Bertin, 1982]. These studies suggest that there is a threshold seed number below which it is not advantageous for the plant to mature fruits.
In addition, in plants with self-incompatibility (SI), when a pollen grain produced in a plant reaches a stigma of the same plant or another plant with a similar genotype,

the process of pollen germination, pollen tube growth, ovule fertilization, and embryo development is halted at one of its stages, and consequently no seeds are produced [Sage and Sampson, 2003]. SI is defined as 'the inability of a fertile hermaphrodite seed plant to preduce zygotes after self-pollination'. It is a general name for several genetic mechanisms in angiosperms, which prevent self-fertilization and thus encourage outcrossing. In some species, fruits from self-pollinated flowers tend to have fewer seeds and are more likely to abort than fruits from cross-pollinated flowers [Murneek, 1954].

Assimilate avalilability

The variation in number of pods and seeds highly depends on their access to assimilates [Arathi et al., 1996; 1999; Bawa and Webb, 1984; Lee and Bazzaz, 1982; Stephenson, 1980]. The assimilate availability for one organ depends both on the quantity of assimilates available at the whole plant level [Hocking and Pate, 1977] and on the competition with the other demanding organs [Hocking and Pate, 1977; Robinson et al., 1980]. These processes are subject to different constraints. Plant architecture has a strong effect on assimilate partitioning among organs [Farrington and Pate, 1981]. Flower position within one inflorescence and inflorescence position within the overall architecture of the plant are important factors affecting yield variation [Brookes et al., 2010; Ellis and Sedgley, 1992]. Within one inflorescence, flowers and seeds located close to the source of assimilates are more likely to survive [Diggle, 1995; Guitián and Navarro, 1996; Medrano et al., 2000; Nakamura, 1986; Thomson, 1989]. This phenomenon is observed in many species [Berry and Calvo, 1991; Diggle, 1997; Obeso, 1993]. The number of reproductive structures that depend on the available resources also affects the allocation of assimilates between flowers or fruits located on different branches [Keiller and Morgan, 1988; Stephenson, 1981].
The course of fruit development in flowering plants includes periods of considerable overlap between growing fruits and seeds among inflorescences. This pattern of intense reproductive growth causes high demand within a short period of time. Thus, the timing of organ initiation and development regulates the partitioning of assimilates in the plant. Furthermore, senescence of the leaves during pod development decreases the assimilate supply, which controls the overlap in the growth of competing sinks and the relation between the photosynthetic source and sink [Bustan et al., 1995]. As a result, early developed fruits and seeds receive more resources than those that develop later [Guitián and Navarro, 1996; Stephenson, 1980; Thomson, 1989].
Based on the factors of variation discussed above, we analysed the yield elaboration in WOSR on both the pod scale and the plant scale, according to the position and time of pod development. Yield elaboration on the pod scale depends on the number of ovules. Fertilisation then influences the number of seeds per pod. Once the number of seeds is set, assimilate accumulation in the seed can lead to an increase in seed weight. At each stage, competition for assimilates results in a reduction in either the number or the weight of the organs. Thus, it is important to study the variations in yield components

and the relationship between their variability and assimilate availability.

Architectural effects

Some authors have proposed that resource competition is not the only cause of differences at the intra-inflorescence level, but rather that developmental constraints [Diggle and Miller, 2004; Wolfe, 1992] or architectural effects [Diggle, 1995; 1997; Pritchard and Edwards, 2005] also have to be considered. Architectural patterns of intra-inflorescence variation may be viewed as the result of natural selection and/or resource conditions among flowers at different positions [Ashman and Hitchens, 2000; Brunet and Charlesworth, 1995; Burd, 1999; Frank, 1987; Mazer and Dawson, 2001]. Architectural variation can mimic the effects of resource competition within inflorescences [Buide, 2008; Stephenson, 1981]. As a result, the importance and/or magnitude of resource competition as a source of variation in plant reproductive characters has likely been vastly overestimated [Diggle, 2003]. According to these hypotheses, differences in floral characteristics from early to late flowers, or from proximal to distal position within the inflorescence, are maintained even in the absence of differential resource allocation, and recent studies have attempted to dissect the causes of intra-inflorescence variation by testing these two hypotheses (i.e. extrinsic resources vs. intrinsic restraints; note of course that both effects may act simultaneously) [Ashman and Hitchens, 2000; Diggle, 1995; Medrano et al., 2000; Wolfe and Denton, 2001].

Flower position within inflorescences and inflorescence position within the overall architecture of the plant are important factors resulting in abortion of pods and seeds [Hiei and Ohara, 2002; Medrano et al., 2000; Stephenson, 1981], which is connected to the effect of flowering time [Hiei and Ohara, 2002]. One hypothesis for a proximate cue is that architectural variation is just resource competition occurring earlier in development than the fruiting stage. That is, variation in morphology or function among flowers of non-fruiting plants occurs because resources will always be consumed during the developmental process and later, distal, flowers will always be at a disadvantage with respect to resource allocation. A second hypothesis is that architectural effects are attributable to declining amounts of vascular tissue along the length of an inflorescence [Wilson, 2001]. The same complex patterns of intra-inflorescence variation that argue against resource variation also indicate that vascular supply may not provide a universal explanation. In addition, vascular differentiation is clearly plastic and responds to the demands of developing sinks such as flowers and fruits [Bustan et al., 1995; Ganeshaiah and Uma, 1994; Preston, 1998]. Inflorescences are likely to contain the amount of vascular tissue necessary to supply the demands of distal structures.

Architectural effects are a common and important component of plant reproductive phenotypes and understanding positional variation is critical to many different areas of plant reproductive biology.

According to the analyses above, the following questions were addressed: Do the numbers of ovules per ovary, seeds per pod and pollen grains per stigma vary with pod

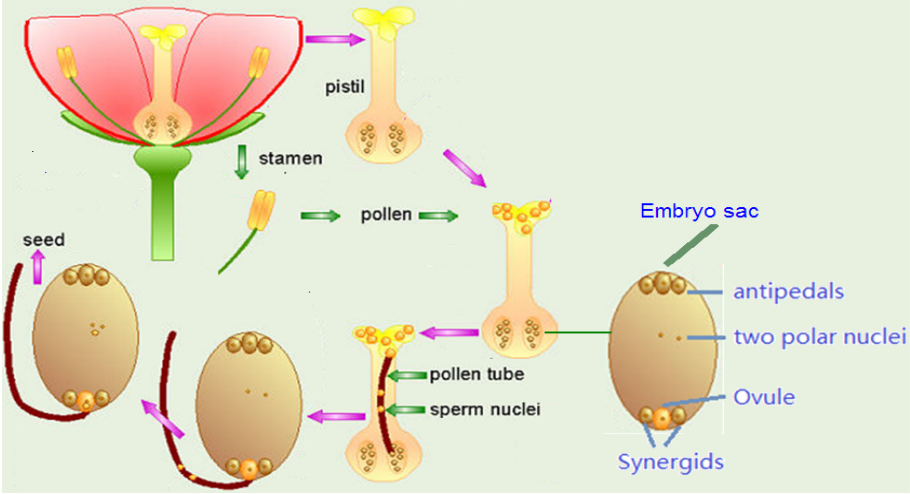


Figure 1.1: Life cycle of flowering plants and the structure of embryo sac (From website). Flower is composed of four parts: calyx, sepal, stamen and pistil. The stamen produces pollen and the ovary includes the embryo sac with ovules

position and the time of appearance? If so, are the variations correlated to the assimilate competition or to pollination limitation?

1.2.2 Biology of flower fertility in flowering plants

In flowering plants, most angiosperms have hermaphrodite flowers, which can produce both male and female gametes (Fig. 1.1). Female gametes are produced inside the ovary of the flower, where they remain until they are fertilized. In some types of flower, the ovary may hold as many as a hundred female gametes, each one contained inside a separate ovule. The series of cell divisions leads to the production of a female gamete inside an ovule. The sequence begins with a hypodermal cell, which is just one of the normal, diploid cells that make up the ovule. After one meiotic division and three mitotic divisions, this produces a structure called the embryo sac which contains eight haploid nuclei, one of which is the ovule, or female gamete. By the end of the maturation process, every ovule in the ovary of the flower will contain a single embryo sac with a single ovule, or female gamete, inside it [Johnstone, 2000].
The production of male gametes, in the anthers of the flower, involves a slightly different series of cell divisions [Johnstone, 2000]. As before, the sequence starts with a hypodermal cell, which is just one of the normal, diploid cells that make up the anther. This hypodermal cell undergoes a single meiotic division to produce four haploid daughter cells. In male gamete production, in contrast to female, all of these daughter cells survive and each one undergoes a further mitotic division to produce a pollen grain

containing two identical haploid nuclei, one of which, the generative nucleus, is the male gamete. It is important to note that the pollen grain is the structure that contains the male gamete, in the same way that the embryo sac contains the female gamete. The pollen grain is not, itself, a gamete.
The function of the pollen grain is to carry the male gamete from the flower where it was produced to the flower of another plant where it will be able to fertilize a female gamete. The transfer of pollen from the anther of one flower to the stigma of another is known as pollination. When an insect visits the flower it gets dusted with sticky pollen which will then get rubbed off on the stigma of the next flower it visits. Other flowers, such as those found on a wheat plant, are wind-pollinated. They produce large quantities of very light pollen grains which are carried away by the wind and picked up by the dangling, feathery stigmas of other wind-pollinated flowers.
Once a pollen grain has landed on the stigma of a compatible flower, it germinates and produces a pollen tube which begins to grow down through the style of the flower towards the ovary. The growth of the pollen tube is controlled by the tube nucleus. The generative nucleus, or male gamete, travels down the pollen tube just behind the tube nucleus and in this way it is able to get down to an ovule, inside the ovary of the flower, where it can fuse with a female gamete.
Once the pollen tube has reached an ovule, it stops growing, and the tube nucleus, having done its job, breaks down. The generative nucleus then undergoes a final mitotic division and splits in half to produce two male nuclei. One male nucleus fuses with the ovule, or female gamete, at the bottom of the embryo sac, to produce a diploid zygote. The other male nucleus fuses with the two polar nuclei, in the middle of the embryo sac, to produce a triploid endosperm nucleus. This double ferti1ization is unique to flowering plants.
After fertilization, the ovule develops into a seed. The zygote grows to become the embryo, or baby plant, inside the seed, while the triploid endosperm nucleus divides away repeatedly to produce the seed's endosperm food store [Reiser and Fischer, 1993]. The integuments, which once surrounded the ovule, become the seed's coat and the ovary, in which the seed is located, swells up to become a fruit [Johnstone, 2000].
Flowers are described as reproductive organs because they are the place where male and female gametes are produced [Johnstone, 2000]. The function of the flower is to ensure fertilization of the ovule and development of fruit containing seeds. More typically, the flower-bearing portion of the plant is sharply distinguished from the foliage-bearing or vegetative portion, and forms a more or less elaborate branch-system called an inflorescence.
An inflorescence is a group or cluster of flowers arranged on a stem that is composed of a main branch or a complicated arrangement of branches. Inflorescences are described by many different characteristics including how the flowers are arranged on the peduncle, the blooming order of the flowers and how different clusters of flowers are grouped within it.

1.2.3 Models of flower fertility

Based on the analysis above, to improve the seed produciton, it is important to study the processes of flower fertility for flowering plant and the factors influencing the reproductive success.
According to the biological description, seed production involves several processes and floral components, as mentioned before. The association of ovule and pollen is likely to create a seed but in some conditions there may be an abortion of the seed due to a fertility problem [Arathi et al., 1999]. Thus, we can simplify the production of a seed as the combination of several physiological processes, namely formation of ovules and pollen grains, fertilization of the ovules, development of young embryos and pods, any problem in these processes may result in seed abortion or pod abortion. Our objectives are firstly to develop a probabilistic model to simulate the number of seeds per pod by reproducing the processes of flower fertility and validate the model using the experimental measurements; secondly, by conducting the different treatments, to identify the factors of influencing the development of seeds and pods.
Several models were developed to model the processes of seed reproduction. A model of seed formation was developed by De Reffye for tropical crops such as coffee, cacao and oil-palm [De Reffye, 1974]. De Reffye (1974) firstly presented a fertility model of coffee flowers. Two main factors impact the yield of coffee. One is production capacity based on morphological characteristics (the number of flowering nodes, the number of flowers per node). The other one is the fertility of flowers. The ovary in a coffee flower contains two ovules. He assumed that the two ovules in the ovary were fertilized by two pollen grains and were independent genetically. Three types of cherry F, A' and B' may be obtained (Fig. 1.2). The fertility probability of one ovule was denoted by $P1$, which was the rate of appearance of a young endosperm from an ovule. A binomial distribution can describe this process. Six to eight months after flowering, the endosperm contained

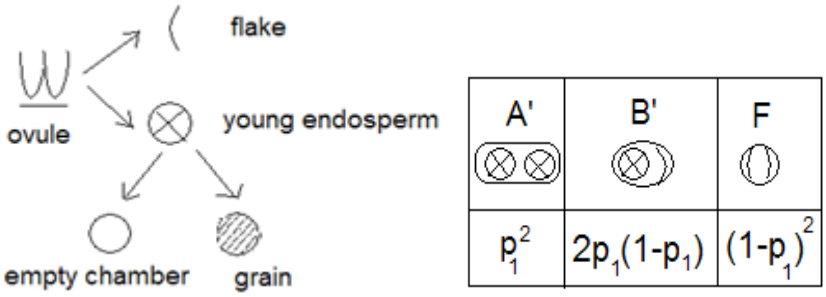


Figure 1.2: Biological description and mathematical expression of ovule fertility in coffee tree

in the boxes of cherries will develop into grains or abortion. The aborted grain gives

the empty chamber. They described the various categories of cherry and ovary by the letters A, B, C, D, E, F (Fig. 1.3).

- Type A: Cherry has two natural grains.
- Type B: Cherry has a grain, and a shell (egg early abortion).
- Type C: Cherry has a normal grain and empty box (endosperm abort later).
- Type D: cherry has an empty box and a shell (empty box peaberry).
- Type E: Cherry has two empty boxes.
- Type F: Ovary not become into fruit and dries in two months (cherry has two shells).

Only categories A, B, C provide grains. The probability $P2$ was used to describe the processes of seed production (Fig. 1.3), which is the rate of appearance of a seed. And another parameter r was used to consider the influence of environmental conditions. Therefore, the probability of an ovule developed into a seed can be computed using the

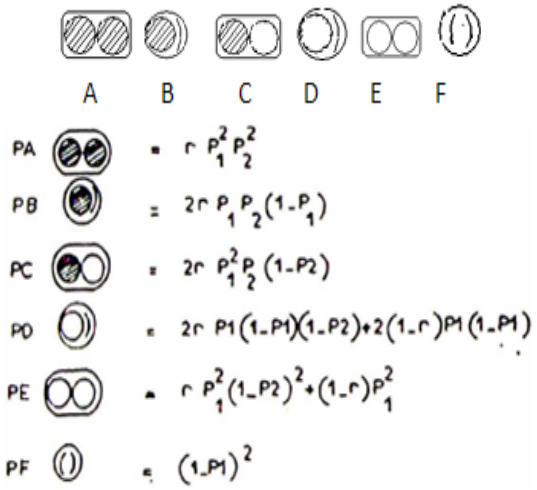


Figure 1.3: Probability of an ovule developed into a seed in coffee tree

equation shown in the fig. 1.3. The result demonstrated that the viability of ovules and of embryos is determined genetically in coffee tree, and they determine, in their turn, the number of seeds. The sterility of flowers is mainly due to the failure of the fertilization of the endosperm in the double fertilization.
Parvais et al. [Parvais et al., 1977] counted the frequency of aggregates of pollen grains and derived the curves representing the law of aggregation of pollen grains for a cacao

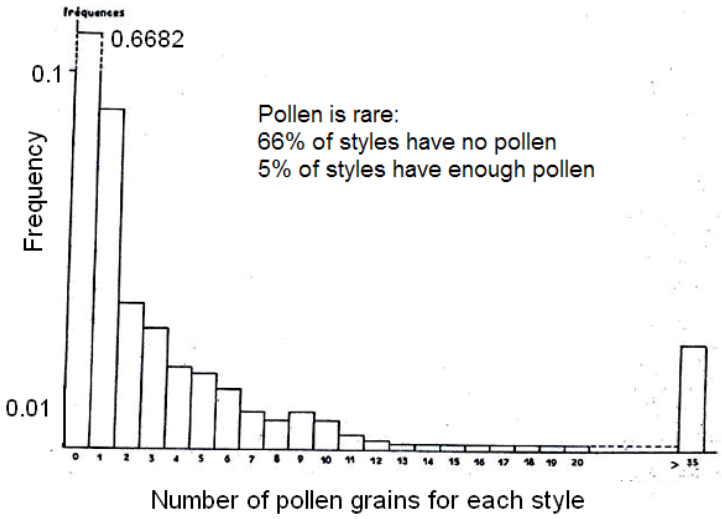


Figure 1.4: Distribution of the number of pollen grains per style in cacao tree

tree. They found that most of pollen grains are singly scattered (Fig. 1.4). Aggregates for more than fifteen of pollen grains are infrequent. The shapes of these curves are always the similar and appears to be a characteristic of cacao tree. Thus, they elaborated a stochastic model of the distribution of number of pollen grains on each style taking account of the distribution of number of ovules per ovary, and compared the simulation with observation. The model is the same as for coffee. Moreover, the variation of the pollination rate among seasons was proven to be closely related to the cacao yield in the fields [Falque et al., 1995; Mossu et al., 1981]. The scarcity of effective pollinators and the low frequency of large aggregates of pollen grains explained the low percentages of pollinated flowers in cacao.

To assess the proportion of different factors controlling the seed production, De Reffye et al. [de Reffye et al., 1978] represented the stochastic processes of flower fertility in cacao tree. They took into account four parameters, including the variability in the number of ovules per ovary N (normal distribution), the condition of pollination a (Pareto distribution), the rate of formation of ovules to seeds P (Binomial distribution) and the wilting point of a pod Xw. They measured the number of ovules per ovary, the number of pollen grains per style and the number of seeds per pod and compared them with the computed values (Fig. 1.5). The results indicated if the number of ovules per ovary (N), the rate of formation of ovules and seeds (P) and the wilting point Xw are fixed, the yield does depend only of the quality of pollination, that is to say, the effectiveness of pollen, the total number of pollinated flowers, the scarcity index of pollination. These last two parameters are very variable even for a same genotype. The model is the same as for coffee. However, the model relies more on simulation tech-

niques. Estimation of parameter values was empirical. Similar results were obtained on the oil-Palm [Lecoustre and De Reffye, 1987]. Another model was developed for kiwifruit [Lescourret et al., 1999].

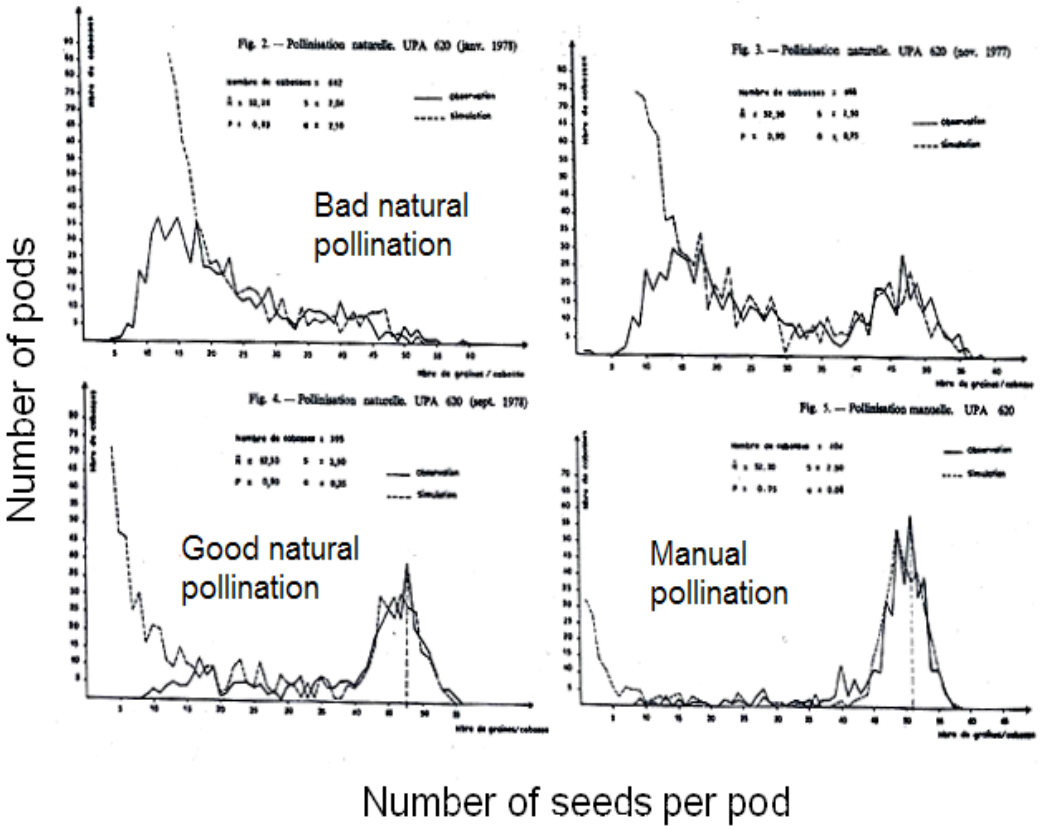


Figure 1.5: Observed and estimated values of the number of seeds per pod in cacao tree under different pollination conditions

1.2.4 Developmental characteristics of WOSR

In WOSR, the life cycle can be divided into seven stages: germination/emergence, production of leaves, extension of the stem, development of buds, flowering, pod development and seed development [Sylvester-Bradley and Makepeace, 1984]. In particular, the latter three stages overlap considerably, because vegetative, generative and reproductive organs develop simultaneously [Diepenbrock, 2000], which lead to the variation of the yield.
Winter oilseed rape have a complex branched structure, after initiation of the inflores-

cence on the main stem, the apices of branches turn also into inflorescence. Although the initiation of branches starts from base to top, the flowers bloom in the inverse order of the initiation of their branches. Besides, flowers develop acropetally on each inflorescence, and the flowering ceases at about the same time on all inflorescences [Keiller and Morgan, 1988]. Hence, this 'double sense' gradient induces large differences in age and position of pods within the inflorescence/plant and hence in pods access to assimilates during their development [Tayo, 1974; Tayo and Morgan, 1975]. WOSR is appropriate to investigate resource investment and pollination between individual flowers and inflorescences and subsequent seed production. As the developmental patterns of floral organs (flower position, flower size, flower number) [Takahata et al., 2008] impact the number of seeds, regarding floral biology, it is an interesting species bearing several inflorescences with more than forty flowers per inflorescence. Besides, flowering lasts about one month inducing a large extension of time between the first emerged pods and the last ones, and therefore, they are subject to contrasting environmental conditions. The ultimate number of pods and seeds is very dependent on a continuous supply of assimilates [Diepenbrock, 2000].

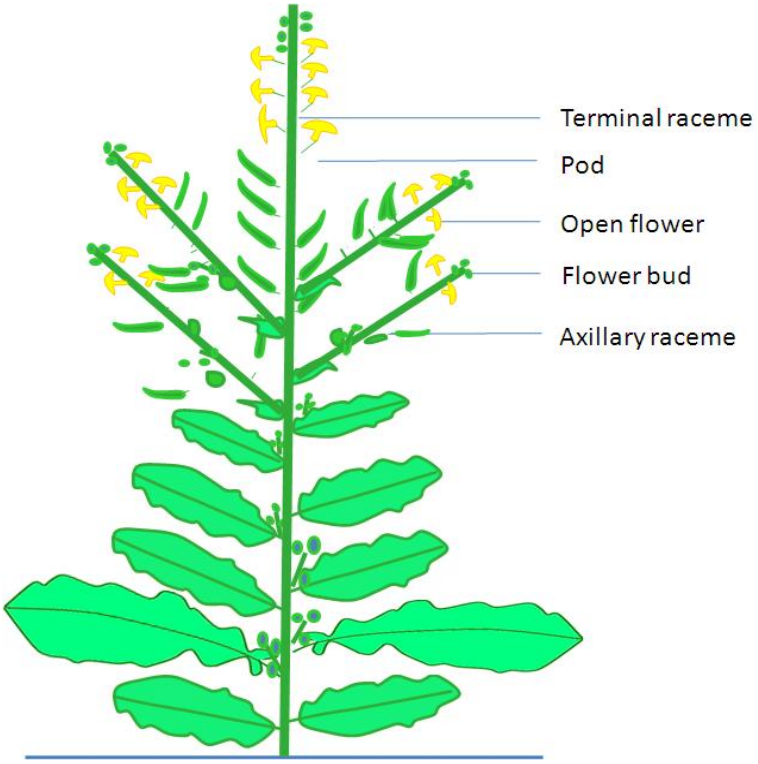


Figure 1.6: Structure of Oilssed rape

1.3 Presentation of the approach

Our main objective was first to analyze the variability of yield components and distinguish the factors of influencing the reproductive failure by the analysis of experimental data. The factors includes ovule viablity, pollination limitation, assimilate competition and architectural effects. Secondly, according to the biological description of flower fertility, we developed a probabilistic model to describe the processes of flower fertility and estimated the model parameter values using the experimental data with Genelized Least Square method (GLSQR). Then we analyzed the stability of model parameter using resampling method and tested if the model can be used to simulate the other flowering plants.

To perform the objectives presented above, we introduced the experimental designs in WOSR in the Chapter 2. we first made some experiments in WOSR to analyze the characteristics of the numbers of ovules per ovary and of seeds per pod, and the number of pods per axis. Then we carried out some treatments including clipping of main stem or ramifications and removal of early flowers to decrease the competition of assimilates. Accordingly, we analyzed the effect of assimilate competition on the number of ovules per ovary, the number of seeds per pod and the number of pods per axis/plant. Furthormore, to analyze the difference of the number of ovules per ovary and of the number of seeds per pod among varieties, we made the measurements for four varieties. In the Chapter 3, we analyzed the effect of different factors on the numbers of ovules per ovary, seeds per pod and pods per inflorescence. These factors include pod position (within one inflorescence and between inflorescences), the time of appearance, pollination conditions and assimilate availability. Furthermore, the differences of the number of ovules per ovary and seeds per pod between varieties were studied. We presented a model using some probabilistic distributions to compute the number of seeds per pod in the Chapter 4. The model can simulate the distribution of the number of pollen grains and distinguish the factors influencing seed production. The model parameter were estimated using the nonlinear parameter estimation method (GLSQR). We estimated the parameters of model between two years (2008 and 2009) using the model. The differences of parameter values between the two years were analyzed. In addition, to analyze the effect of assimilate availability on the number of ovules per ovary and the number of seeds per pod, we estimated the parameters between the control and clipped plants (Clipping of main stem or removal of early flowers). As mentioned in the introduction, the flowering time and flower position has influence on the number of seeds per pod, we estimated the parameters according to the pod rank on the main stem and between inflorescences to analyze the effect of plant architecture. Furthermore, the parameters were estimated for four varieties to analyze the difference of the yield components. In the following part, we studied the stability of the model by using the random subsets methods, including bootstrap resampling and jackknife resmapling. We compared the model in the thesis with the model of flower fertility developed by Lescourret et al. in

kiwifruit. The simulation of the number of pollen grains was improved by introducing a parameter k (the proportion of effective pollen grains). In the last, the model was used to compute the number of seeds per pod in other flowering plants, such as soybean and cacao tree.

Part I

Experiment Designs
Results of Experiment

Chapter 2

Field experiment approach

2.1 Plant materials

WOSR is an annual plant with inflorescences of yellow flowers. Seeds are sown in the autumn and before winter. The plant develops a rosette of 10-15 leaves [Jullien et al., 2010]. Stem extension begins with the return of the growing season in the spring. Flowering begins before stem extension has finished and continues for more than one month. The number of ramifications is pre-determined during the organ initiation early in the growth cycle in autumn. The meristem produces leaves, which bears axillary buds that can produce a ramification [Diepenbrock, 2000; Tittonel, 1990].
Flowering begins with the opening of the lowest bud on the main stem and continues upward with three to five or more flowers opening per day. Flowering at the base of the first secondary branch begins two to three days after the first flower opens on the main stem. WOSR has entomophilous flowers that are capable of both self- and cross-pollination. [Becker et al., 1992] found that depending on variety and weather, oilseed rape exhibits approximately 30% out-crossing. Insect mediated cross pollination may be of only secondary importance for oilseed rape [Mesquida et al., 1988]. Following emergence of the leaves, internodes of the main stem begin to elongate. The expansion of the ramification is delayed compared to the main stem. Lateral inflorescences expand along the main stem from the top to the bottom. Flower emergence starts on the main inflorescence and develops basipetally to the lateral inflorescences [Tittonel, 1990], causing the basal and oldest ramifications to bear the youngest inflorescences. Pods are set once all of the leaves of the main stem have emerged. The first pods are initiated on the main stem and then on the ramifications from apical to basal. Within a ramification, pod setting remains acropetal [Jullien et al., 2010].
The inflorescences were numbered from top to bottom along the main stem. Thus the main stem is number R0 and the highest ramification is number R1. Flowers and pods on each inflorescence were recorded by their rank number starting from the base of the inflorescence (Fig. 2.1). On an inflorescence, pod number 1 is closest to the leaves and the main stem.

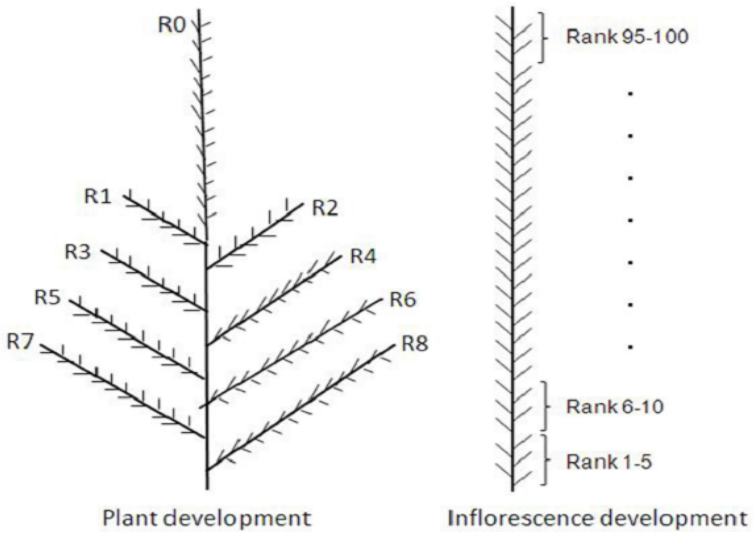


Figure 2.1: Schematic diagram of winter oilseed rape. Plant development: the inflorescences initiate acropetally but expand basipetally. Flowering starts on the main stem (R0) and is followed by lateral inflorescences from top to bottom (basipetal). Inflorescence development: flowering and pod setting on the inflorescence occur from bottom to top (acropetal)

2.2 Experimental design and growing conditions

Field experiments were conducted in Grignon (velines, France, 48.9◦ N, 1.9◦ E) at the National Institute for Agricultural Research (INRA) in 2008 and 2009. Seeds were sown on September 9th in the two seasons at a density of 50 seeds per m^2. Plots were twenty rows, 0.30 m apart and 30 m long, and the plots were kept free of weeds, insects and diseases. The plants were harvested at the beginning of July.

2.3 Measurements of number of ovules per ovary and seeds per pod on the main stem

The experiment was conducted in 2007-2008 and used the Mendel variety. To study the characteristics of the number of ovules per ovary and the number of seeds per pod, the abortion rate of pods, we randomly selected 45 plants to count the number of ovules per ovary and seeds per pod according to their rank on the main stem. The number of ovules per ovary was calculated as the sum of mature and aborted seeds per pod. Measurements started when all the flowers have developed into pods to make sure that

the number of seeds and ovules can be measured in all the pods of the inflorescences. The experimental data was used to calibrate the model paramters and analyze the effect of pod rank on the main stem.

The experiment was conducted in 2008-2009 used the Mendel variety. 18 plants were randomly collected to compare the estimated parameters among years. The number of seeds and aborted ovules per pod for all the pods on the main stem were counted according to the pod rank. In addition, the number of seeds and aborted seeds on the ramification R1, R4, R7, R9, R11 were measured according to their positions on the inflorescences. Accordingly, we can analyze the difference of parameter values between inflorescences. The experimental data of the two years were used to estimate the model parameter values and to analyze the difference of parameter values between years. Analysis of variance (ANOVA) was used to test the effect of pod position and the number of ovules per ovary on the number of seeds per pod.

2.4 Difference of the number of ovules in different developmental stages

We observed the number of ovules per ovary in different developmental stages for flower buds, flowers and pods (Fig. 2.2) to study the difference of the number of ovules per ovary between flower buds, flowers and pods in 2008-2009. The variety was Pollen. One plant was randomly selected to count the number of ovules per ovary with Microscope. We chose the pods on the main stem and ramifications R1 and R4 to analyze the difference between different inflorescences. The measurements were conducted five times during the flowering period and one plant was measured for each time.
ANOVA was performed to test the difference of the number of ovules between bud, flower and pod for each inflorescence, and the difference of the number of ovules per bud, flower and pod between the inflorescences R0, R1 and R4. Tukey's HSD (Honestly Significant Differences) multiple comparison test was used when significant effect was encountered to determine which means were significantly different from one another.

2.5 Effect of pollination conditions on the number of ovules and seeds per pod

To analyze if pollination condition has an effect on the number of seeds per pod in WOSR, hand pollination was carried out for the Mendel variety in 2008-2009. We randomly selected 24 plants to pollinate the flowers on the main stem. One day before pollination, select the plants to remove the ramifications, opening flowers by tweezers, then isolate them with plastic bags. Four plants were selected as self pollinated plants

Figure 2.2: Ovules in bud, flower and pod in WOSR. A. Bud; B. Flower; C. Pod. Measurement scale: 0.5 mm

(CK_Self). Pick up the anthers with pollen from the other plants and rub the pollen on the stigma of flowers for the rest 20 selected plants. After pollination, put the plastic bag to isolate them and make sure that the bags will not influence the growth of flowers after 2-3 days. Take the bags off after one week. In addition, select 6 plants from the treatment of clipping all the ramifications (M_R-) as the natural pollinated plants (CK_Natural). Because the early flowers were clipped for the hand pollinated and self pollinated plants, the number of seeds and aborted ovules per pod from the pods located rank 20-60 were counted for each plants one month later. The number of ovules per pod was computed as the sum of the number of seeds and aborted ovules. ANOVA was used to test the difference of the number of ovules and seeds per pod between different pollination conditions.

2.6 Effect of pod position and the time of pod appearance on the number of ovules per pod, seeds per pod and pods per inflorescence

On the field experimental site, the plants started to flower in mid-April, and, the flowering season continued until mid-May. To investigate the effect of the position and appearance time of pods on the number of ovules and seeds per pod among inflorescences, 18 Mendel plants were randomly marked in mid-April in 2008-2009, just before the flowering season. When the plants came intobegan to bloom, the numbers and positions of flowers that bloomed within inflorescences were recorded every two or to three days throughout the flowering season from Apr,16^{th}-May,18^{th}. The positions and the times of appearance of the flowers and pods were recorded from the main stem (R0) and from the ramifications (R1, R4, R7, R9 and R11, see Fig. 2.1). The plants were same with the plants measured in the Section 2.3. Furthermore, to analyze the effect

of the number of flowers on the number of aborted ovules, the plants in a plot with the area of 0.65 m^2 ($1.23m \times 0.53m$) were selected to count the number of opening flowers every 2-3 days.
The Kruskal-Wallis rank sum test was used to test the difference in the distributions of ovules and seeds per pod with times of pod appearance.

2.7 Effect of assimilate availability on the number of ovules per pod, seeds per pod and pods per inflorescence

2.7.1 Clipping treatments and measurements

The demand of assimilates can be changed by clipping the main stem, ramifications or basal flowers. Thus, clipping treatments were conducted in 2008-2009 to investigate the effect of the competition for assimilates inter-flower within an inflorescence, among inflorescences and at the plant level. To check if the characteristics of yield components are independent of variety, two varieties were used (Mendel and Pollen). First, 20 Mendel variety plants were randomly selected on the basis of similarity of their developmental stages in the field. Two treatments were administered, clipping the main stem (Treatment M_M-) or clipping ramifications (Treatment M_R-). Clippings were done when there were about 20 flowers on the main stem. The plants selected for the continuous observations were used as the control plants for the Mendel variety (Treatment M_CK). Second, 15 Pollen variety plants were randomly selected on the basis of similarity of their developmental stages. Clippings of 20 basal flowers on the main stem and all of the ramifications (Treatment P_R-') was were conducted for 10 plants. The other 5 plants were used as the control (P_CK). We assumed that if the presence of early opening flowers led to a reduction of late opening flowers due to assimilate competition and that, if this was the principal cause of fruit failure in late opening flowers, then later opening flowers should produce more pods or seeds compared to control plants when released from competition of early flowers. All of the treatments are shown in Table 2.1.
Measurements began in mid-June. Measurements started when all of the flowers had developed into pods to ensure that the number of seeds and ovules could be measured in all of the pods of the inflorescences. For each plant, the number of pods per inflorescence and the number of seeds and aborted seeds per pod were carefully recorded according to the position of the pod (number of inflorescence and rank on the inflorescence). The number of ovules per pod was calculated as the sum of undeveloped ovules, aborted seeds and mature seeds per pod. The seed dry weight per ten pods was measured for the treatment M_R-, M_CK and P_R-' treatments. The experimental data of the treatemts M_CK, M_R-, P_CK and P_R-' were also used to estimate the model

parameters to analyze the effect of assimlate avalability. The pods were gathered five by five to analyse the effect of pod rank on the number of ovules and seeds per pod. Because the length of inflorescences is different between plants, pod ranks were normalised for each inflorescence by dividing by the maximum rank on the inflorescence. This approach allows the conversion of the ranks of all of the inflorescences into a range between 0-1.

Table 2.1: Description of clipping treatments for two varieties 'Mendel' (M) and 'Pollen' (P). For Mendel: M_R- denotes the treatment of clipping all the ramifications; M_M- denotes the treatment of clipping the main stem and keeping all the ramifications; M_CK denotes the control plants (no treatment); For Pollen: P_R-' denotes the treatment of clipping all the ramifications and removing the 20 basal flowers; P_CK denotes the control plants. '+' and '-' represent keeping or removing main stem, ramification or 20 basal flowers respectively.

Variety	Treatments	Main stem	Ramifications	20 basal flowers	Sample size
Mendel	M_R-	+	-	+	10
	M_M-	-	+	+	10
	M_CK	+	+	+	18
Pollen	P_R-'	+	-	-	10
	P_CK	+	+	+	5

2.7.2 Statistial analysis of the effect of pod position and clipping treatments on the number of ovules and seeds per pod

Segmented regression is a method of regression analysis in which the independent variable is partitioned into intervals and a separate line segment is fit to each interval. The boundaries between the segments are breakpoints. Segmented regression is regression analysis in which changes in the mean outcome levels and trends before and after an intervention are estimated [Wagner et al., 2002].
In our study, segmented regression was used to estimate the change in trend in pod rank before and after a breakpoint and the difference between the control and clipped plants. We created several variables to analyse the effect of pod rank and clipping treatment. A created variable is an artificial variable created to represent an attribute with two or more distinct levels.
Our experimental data showed that there are three segments on the main stem, and two segments on ramifications. Therefore, segmented regression with one or two breakpoints was used for ramifications and the main stem, respectively.

- Segmented regression with one breakpoint Segmented linear regression with two segments separated by a breakpoint can be useful to quantify an abrupt change in the response function (Y) of a varying influential factor (X).

$$Y = b_0 + b_1X + b_2T + e_t \tag{2.1}$$

X is the variable of pod rank; T is a created variable for before or after the breakpoint. T is coded 0 before the breakpoint and continuous starting at 1 after the breakpoint; e_t is the random variation at rank X not explained by the model. b_0 is the intercept of the line, b_1 is the slope before the breakpoint and b_2 is the change in the slope before and after the breakpoint (difference in the slopes of two segments).

- Segmented regression with two breakpoints

$$Y = b_0 + b_1X + b_2TA + b_3TB \tag{2.2}$$

X is pod rank from baseline; TA is a created variable for the first segment coded 0 before 1^{st} breakpoint and starts at 1 after the breakpoint; TB is a created variable for the second segment coded 0 before 2^{nd} breakpoint and starts at 1 after the 2^{nd} breakpoint. b_0 is the value of dependent variable at baseline; b_1 is the trend prior to the 1st breakpoint; b_2 is the change in trend after the 1^{st} breakpoint; b_3 is the change in trend after the 2^{nd} breakpoint.

- Segmented regression between two groups with one breakpoint
A dummy variable is incorporated for group to analyse the change in slope after the breakpoint and between the control and clipped group.

$$Y = b_0 + b_1X + b_2T + b_3G + b_4GX + b_5GT \tag{2.3}$$

G is a created variable for groups, coded 0 for control plants and 1 for clipped plants; GX is a created variable for the control plants coded 0 before 1st breakpoint and starts at 1 after the breakpoint; GT is a created variable for the clipped plants coded 0 before the 1^{st} breakpoint and starts at 1 after the breakpoint.
b_0 is the value of dependent variable at baseline; b_1 is the trend before the breakpoint; b_2 is the change in trend after the breakpoint; b_3 is the difference between the groups; b_4 is the difference between the groups in change in trend before the breakpoint; b_5 is the difference between the groups in change in trend after the breakpoint.
The t-test is used to test the significance of the individual coefficients in the equation. For example, if we are testing $H0 : bi = 0$ and $Ha : bi \neq 0$, then we consider the P-values to determine whether to reject or accept $H0$. If the P-value is less than 0.05, then we reject $H0$. The null being tested by this test is $bi = 0$, which indicates that this variable is not related to Y.

2.7.3 Significant tests of the difference of yield components between inflorescence

ANOVA was performed to test the differences of the number of ovules and seeds per pod, the number of pods per axis and the total number of seeds and pods. Tukey's HSD (Honestly Significant Differences) multiple comparison test was used when significant effect was encountered to determine which means were significantly different from one another.
The F-test and Student's t-test were applied to evaluate the differences in the mean numbers of ovules, seeds per pod and pods per axis between control and clipped plants [Stephenson, 1980].

2.8 Measurements of the number of ovules and seeds per pod for four varieties

In 2008-2009, to investigate the difference of the number of ovules and seeds per pod for four varieties (Mendel, Pollen, Exocet and Gamin). 10 plants were randomly selected on the basis of similarity of their developmental stages for each variety. 15 pods from rank 11-40 (to eliminate the effect of position) on the main stem and 10 pods from rank 1-20 on the ramifications were collected on each plant. The number of seeds and aborted ovules per pod were counted. The number of seeds and aborted seeds were counted when all the pods matured. The data were used to analyze the difference of model parameters between varieties.

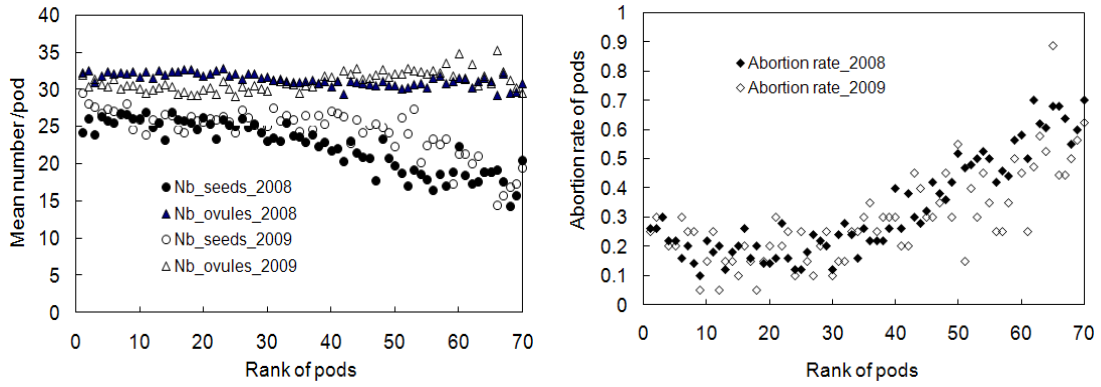
Chapter 3

Field experiment results

3.1 Number of ovules per pod, number of seeds per pod and aboriton rate of pods

The average number of ovules and seeds according to the pod rank on the main stem is given in the Fig. 3.1a, for all the plants measured in 2008 and 2009. For the two years, the number of ovules per pod remained constant along the main stem while that of seeds decreased after the 30th rank.

Flower position had no significant influence on the number of ovules per pod (ANOVA,



(a) (b)

Figure 3.1: Number of ovules per pod, number of seeds per pod and abortion rate of pods according to the pod rank in WOSR.

$P > 0.1$), but it had great impact on the number of seeds per pod (ANOVA, $P < 0.001$). Besides, the number of ovules per pod had no significant influence on the number of seeds per pod (ANOVA, $P > 0.1$) (Table 3.1). The two years experiments gave the similar results (Fig.3.1).

Table 3.1: Analysis of variance for the effects of the number of ovules and positions on the number of seeds per pod.

	Df	$Sum\,Sq$	$Mean\,Sq$	$F\,value$	$P(>F)$
Position	69	8264	120	1.768	0.0002***
Ovule	21	1789	85	1.257	0.196
Residuals	752	50945	68		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ''

Abortion rate of pods on the main stem was a little larger at the beginning, then remained constant and increased linearly with the pod rank (Fig. 3.1b). It seems that the increase in the abortion of pods starts at the same time as the decrease in the number of seeds per pod.

3.2 Difference of the number of ovules in different developmental stages

The study of [Bouttier and Morgan, 1992b] indicated that the conditions experienced by the flower buds over the whole plant when the ovules formed are clearly important in determining eventual seed number per pod.
In our thesis, the number of ovules between bud and flower had no significant difference within one inflorescence (ANOVA, $P > 0.1$, Table3.2), but the number of ovules for buds and flowers had significant difference compared to the number for pods (ANOVA, $P > 0.1$, Table3.2). However, there was no trend for the variation of the number of ovules per flower between the inflorescence R0, R1 and R4, as shown in the fig. 3.2 .
The number of ovules for buds and pods differ significantly between the inflorescences R0, R1 and R4 (ANOVA, $P < 0.05$, Table3.2), but the number of ovules has no difference for flowers (ANOVA, $P > 0.1$, Table 3.2). The decrease of the number of ovules for pods compared to buds or flowers was consistent with the study of [Bouttier and Morgan, 1992b].
The study of stem explants in vitro conducted by [Bouttier and Morgan, 1992a] indicated that open flowers and young pods underwent normal development but the developments of buds was less successful. Young buds (3 mm long) did not develop and only limited development of the older buds (5 mm long) took place. Pod and seed set in open flowers were not affected by adding plant growth substances to the medium. Reducing the supply of minerals to open flowers reduced seed set, pod elongation and pod weight but did not affect pod set. The results showed that the environmental conditions experienced by the flower when it developed are very important.

Table 3.2: Analysis of variance (ANOVA) for the difference of the number of ovules per bud, flower and pod on the inflorescences R0, R1 and R4. Values with different superscripts differ significantly using the Tukey's HSD test at $P < 0.05$. The uppercase letters represent the difference between inflorescences (within a column); the lowercase letters represent the difference between bud, flower and pod (within a row)

	Bud	*Flower*	*Pod*	*P value*
R0	32.2 ± 3.7^{bB}	30.8 ± 2.4^{b}	28.7 ± 3.6^{aAB}	$< 0.0001 * **$
R1	29.1 ± 2.6^{abA}	30.1 ± 3.8^{b}	27.4 ± 3.4^{aA}	$< 0.05*$
R4	32.1 ± 2.6^{bB}	32.1 ± 3.1^{b}	29.9 ± 2.6^{aB}	$< 0.001 * *$
P value	$< 0.01 * *$	> 0.1	$< 0.01 * *$	

Significant codes: 0 '***' 0.001 '**' 0.01 '*' not significant 'ns'

3.3 Effect of pollination conditions on the number of aborted ovules and seeds per pod

Figure 3.3 indicated that the number of ovules did not differ between the hand-pollinated plants (Hand), self-pollinated plants (Self) and natural-pollinated plants (Natural). But the number of seeds per pod was larger on the hand-pollinated plants than that on the self-pollinated plants and natural-pollinated plants. However, the difference of the number of seeds per pod was not significant (Table 3.3). Furthermore, the number of aborted ovules was significantly smaller on the hand-pollinated plants than that on the self-pollinated plants (ANOVA, Table 3.3). The results indicated that the number of seeds per pod did not differ between the hand-pollinated plants and the self-pollinated plants, although the number of aborted ovules was larger on the self-pollinated plants. The results were inaccordance with the study of [Adegas and Nogueira Couto, 1992]. We can conclude that the Mendel variety is self-compatible, the self-incompatible is not the factor leading to the seed abortion.

Table 3.3: ANOVA for the difference of the number of ovules and seeds per pod on the he hand-pollinated, self-pollinated and natural-pollinated plants.

Plants	Number of ovules per pod	Number of seeds per pod	Number of aborted ovules per pod
Hand	31.4±3.0	29.0±4.0	2.3 ± 2.8^{a}
Self	31.7±2.6	27.5±4.8	4.0 ± 4.1^{b}
Natural	31.6±3.4	28.6±3.9	3.0 ± 2.6^{a}
P value	> 0.1	> 0.1	$< 0.01^{**}$

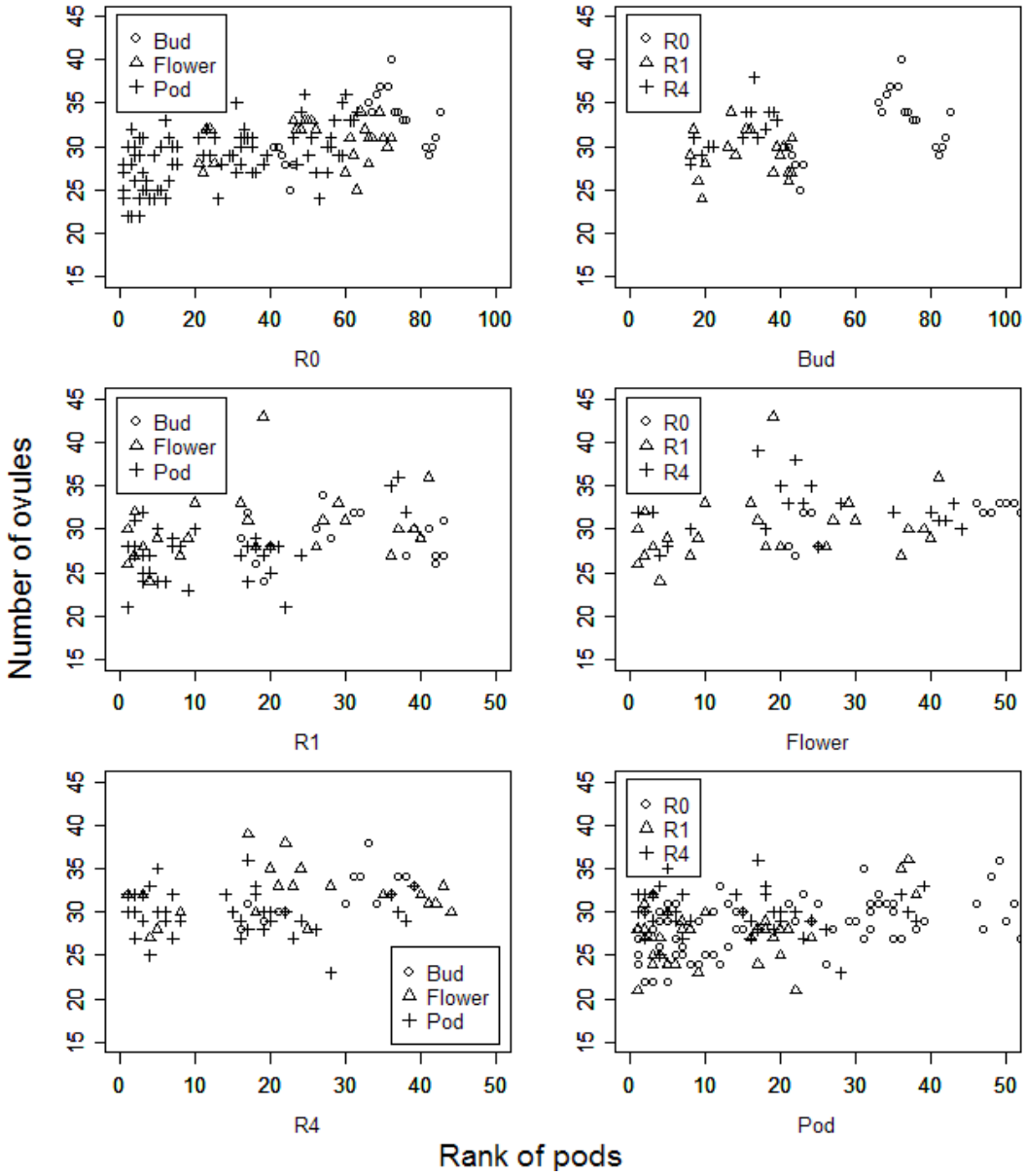


Figure 3.2: Number of ovules in different development stages (Bud, flower and pod) for the inflorescences R0, R1 and R4 (Left column); Number of ovules in inflorescences R0, R1 and R4 for different development stages (Right column). The data were observed by microscope.

3.4 Effect of flower position and time of pod appearance on the number of ovules and seeds per pod

Along an inflorescence axis, pods appear acropetally, pods with higher ranks appear after pods with lower ranks. Furthermore, ramifications grow basipetally (from the top

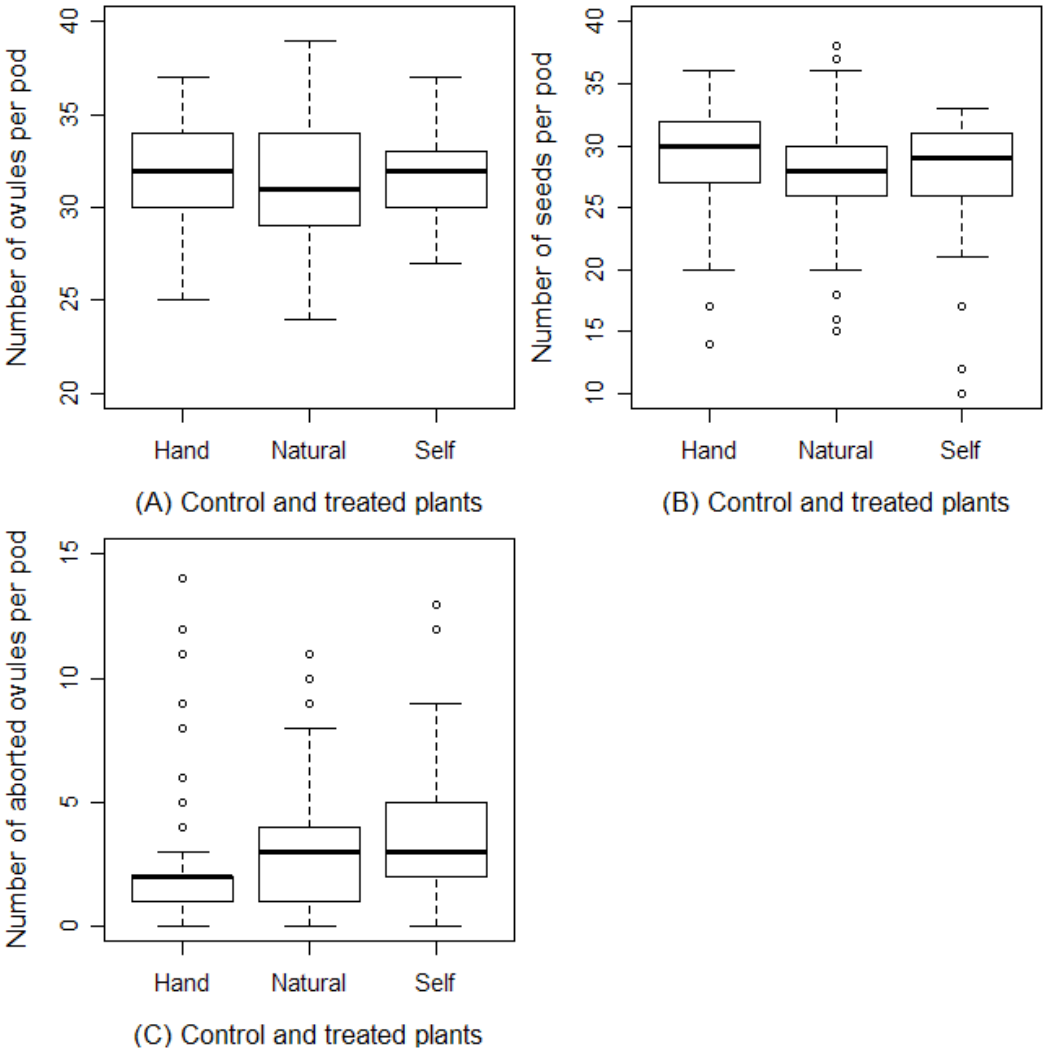


Figure 3.3: Boxplot of the number of ovules per pod (A), seeds per pod (B) and aborted ovules (C) for the hand-pollinated, self-pollinated and natural-pollinated plants (Variety: Mendel).

to the bottom along the main stem). A high correlation exists between pod position and time of appearance for each ramification (correlation coefficient is equal to 1 for each inflorescence on each plant). Fig. 3.4 showed that the mean pod rank increased with the appearance time of pods for each ramification.

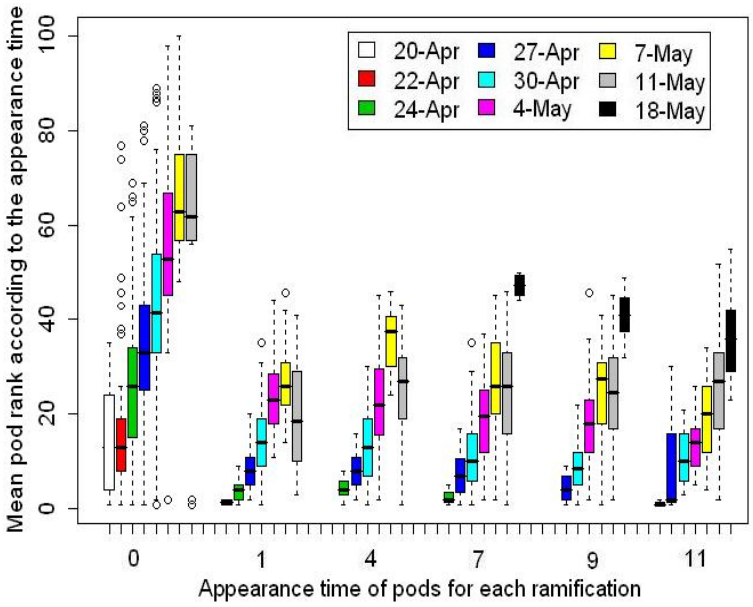


Figure 3.4: Boxplot of pod position and its appearance time for the inflorescences R0, R1, R4, R7, R9 and R11.

3.4.1 Effect of the time of pod appearance on the number of ovules and seeds per pod

Number of ovules and seeds per pod

To analyze the effect of the time of pod appearance on the number of ovules and seeds per pod, the pods located three positions were selected for the inflorescences R0, R1, R4, R7, R9 and R11. The three positions were the basal positon (Normalized rank 0.01-0.1), middle position (rank 0.51-0.6) and distal position (rank 0.91-1).
The mean number of ovules (ANOVA, $F < 1, P > 0.1$ for each ramification) and seeds (ANOVA, $F < 1, P > 0.1$ for each ramification) per pod for the pods located at the basal, middlle and distal positions did not differ significantly according to the time of pod appearance for the inflorescences R0, R1, R4, R7, R9 and R11 (Fig. 3.5). The results indicated that the time of pod appearance had no influence on the number of ovules per pod for these pods.

The distributions of the number of ovules per pod tended to shift towards greater values with the time of pod appearance on the inflorescences R0, R1 and R4 (Kruskal-Wallis rank sum test, R0: $\chi^2 = 88.5, df = 8, P < 0.001$; R1: $\chi^2 = 43.6, df = 6, P < 0.001$ and R4: $\chi^2 = 25.9, df = 5, P < 0.001$). However, it had no significant difference on ramifications R7, R9 and R11 (Kruskal-Wallis rank sum test, R7: $\chi^2 = 7.9, df = 6, P = 0.25$; R9: $\chi^2 = 3.9, df = 5, P = 0.56$; R11: $\chi^2 = 9.2, df = 6, P = 0.17$, Fig. 3.6).
The distributions of the number of seeds per pod showed a statistically significant difference with the time of pod appearance for inflorescences except R11 (Kruskal-Wallis rank sum test, R0: $\chi^2 = 51.3, df = 8, P < 0.001$; R1: $\chi^2 = 32.5, df = 6, P < 0.001$; R4: $\chi^2 = 27.1, df = 5, P < 0.001$; R7: $\chi^2 = 34.7, df = 6, P < 0.001$; R9: $\chi^2 = 43.1, df = 5, P < 0.001$; R11: $\chi^2 = 4.9, df = 6, P = 0.55$). At the end of the flowering time, more pods with a small number of seeds were present.

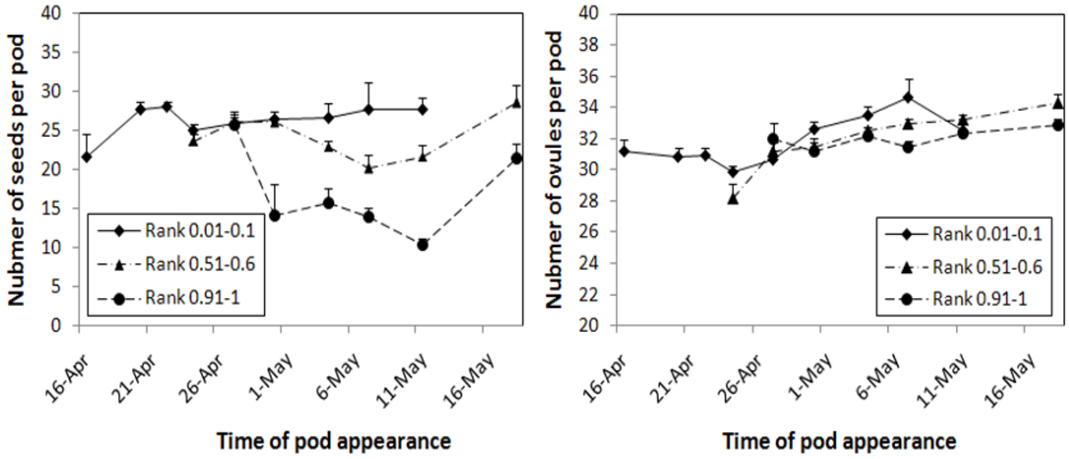


Figure 3.5: Variation of the number of ovules and seeds per pod for the pods located at the basal, middle and distal positions according to the time of pod appearance

Number of aborted ovules per pod

The number of aborted ovules corresponds to the difference between the number of ovules and the number of seeds in a pod. This number of aborted ovules was related to the number of flowers per square meter in the field. The higher the number of flowers in the field was, the smaller the number of aborted ovules per pod (Fig. 3.7). The total number of aborted ovules was large at the beginning then remained constant and increased with the time at the end of flowering. The result indicated that the time of pod appearance influences the number of seeds per pod in pods appears earlier or later during the flowering period.

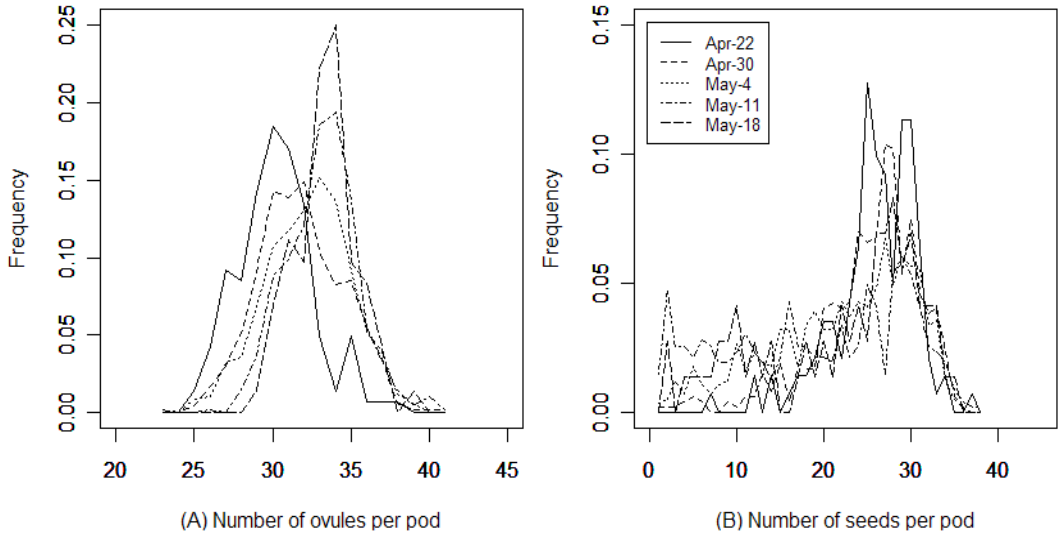


Figure 3.6: Variation of the distribution of the number of ovules and seeds per pod according to the time of pod appearance

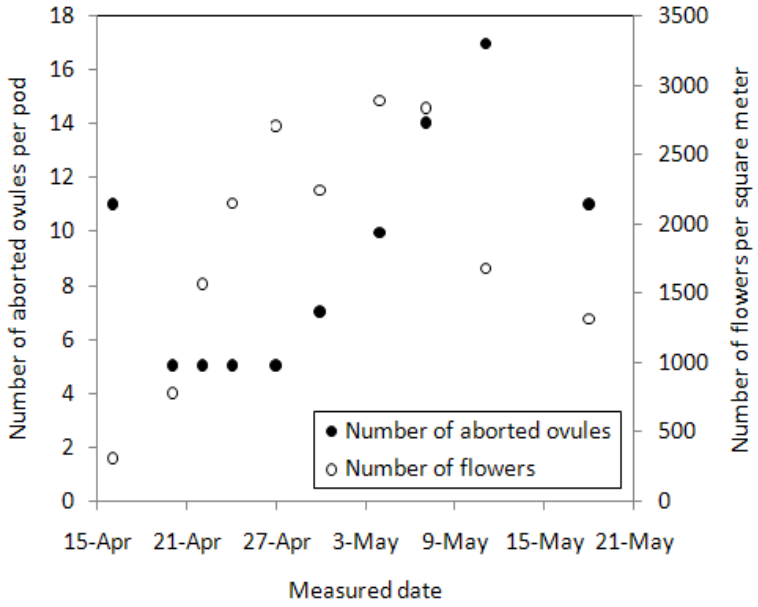


Figure 3.7: Number of flowers in the field per square meter and number of aborted ovules per pod during the flowering period.

3.4.2 Effect of pod position on the number of ovules and seeds per pod

The number of ovules per pod was small on the basal positions, then increased with the pod rank and remained constant on the main stem R0 and the ramifications R1 and R4. But the number of ovules per pod did not vary with the pod rank on the ramification R7, R9 and R11 (Fig. 3.8). In addition, the mean number of ovules per pod increased with ramification rank along the main stem.
The number of seeds per pod remained constant at the basal positions, then decrease with the pod rank for each inflorescence. However, as shown in the Fig. 3.8B, it seemed that the mean number of seeds per pod did not vary with ramification rank, the difference resulted from the pod rank.

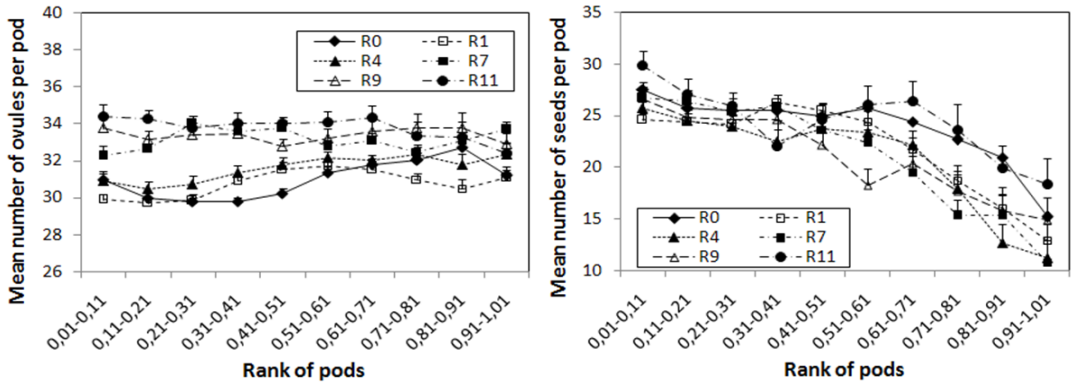


Figure 3.8: Effect of pod rank on number of ovules per pod (A) and number of seeds per pod (B) for different inflorescences; R0, R1, R4, R7, R9, R11 represent the rank number of ramifications downwards.

3.5 Effect of assimilate availability on yield components

3.5.1 Effect of clipping the main stem or ramifications on the number of ovules per pod

Effect of pod position and clipping treatments on the main stem

Effect of pod ranks The segmented regression demonstrated that the number of ovules per pod differed significantly with the pod rank on the main stem in the control plants (coefficient b_1 in Eqn. 2.2, $t = -2.2, P = 0.04$), but not in the clipped plants

(coefficient b_1 in Eqn. 2.2, $t = -1.6, P = 0.13$). The number of ovules per pod fluctuated along the main stem and decreased for a few ranks followed by a tendency to increase and then to decrease at the end of the stem.

Effect of clipping ramifications Significant changes were present in trend (slope) before and after the 1_{st} breakpoint (coefficient b_2 in Eqn. 2.2, 0.19 ± 0.04 for M_CK, $t = 2.9, P < 0.05$ and 0.15 ± 0.09 for M_R-, $t = 2.3, P < 0.05$) and 2nd breakpoint (coefficient b_3 in Eqn. 2.2, 0.85 ± 0.06 for M_CK, $t = -4.5, P < 0.001$ and 0.81 ± 0.06 for M_R-, $t = -2.5, P < 0.05$) in the control and clipped plants, as shown in Fig. 3.9. The number of ovules per pod was significantly different in the second segment between the control and clipped plants (coefficient b_3 in Eqn. 2.3, $t = 3.7, P < 0.001$), but not in the first and third segments. Because the 1^{st} breakpoint was the location where clipping ramifications were performed, the result indicated that clipping ramifications did have an instant influence on the number of ovules per pod on the main stem.
The mean numbers of ovules on the main stem were larger in the clipped plants than in the control plants (t-test, $t = -7.4, df = 379, P < 0.001$). The total mean number of ovules per axis increased ranging from 1340 ± 334 (mean $\pm$ SD) to 1876 ± 455 (mean $\pm$ SD) in the control and clipped plants, respectively.

Effect of pod position and clipping treatments between ramifications

Effect of pod ranks Segmented regression indicated that the number of ovules per pod varied with the pod rank on ramifications R1 (coefficient b_1 in Eqn. 2.1, $t = 3.4, P < 0.01$) and R4 ($t = 4.6, P < 0.001$) in the control plants, but not in the clipped plants (coefficient b_1 in Eqn. (1), $t = -0.6, P = 0.58$ for R1 and $t = -0.3, P = 0.8$ for R4, respectively). The number of ovules per pod did not vary with the pod rank on ramifications R7, R9 and R11 (coefficient b_1 in Eqn. 2.1, $P > 0.1$ for each ramification).

Effect of clipping the main stem Significant differences were present in trend observed for the number of ovules per pod after the breakpoint in the control plants on ramifications R1 (coefficient b_2 in Eqn. 2.1, $t = -4.1, P < 0.001$) and R4 ($t = -3.0, P < 0.05$), but not in the clipped plants (coefficient b_2 in Eqn. 2.1, $t = 1.6, P = 0.14$ for R1 and $t = 0.5, P = 0.66$ for R4). The number of ovules per pod was somewhat small before the breakpoint, and then increased after the breakpoint for ramifications R1 and R4 (Fig. 3.9). However, segmented regression for ramifications R7, R9 and R11 indicated that there were no significant changes in the trend after the breakpoint in the control and clipped plants (coefficient b_1 in Eqn. 2.1, $P > 0.01$ for ramifications R7, R9 and R11).
The mean number of ovules per pod was significantly higher in the clipped plants than in the control plants for the ramifications R1, R4, R7, R9 and R11 (Fig. 3.10, coefficient b3 in Eqn. 2.3, $P < 0.001$ for each ramification).

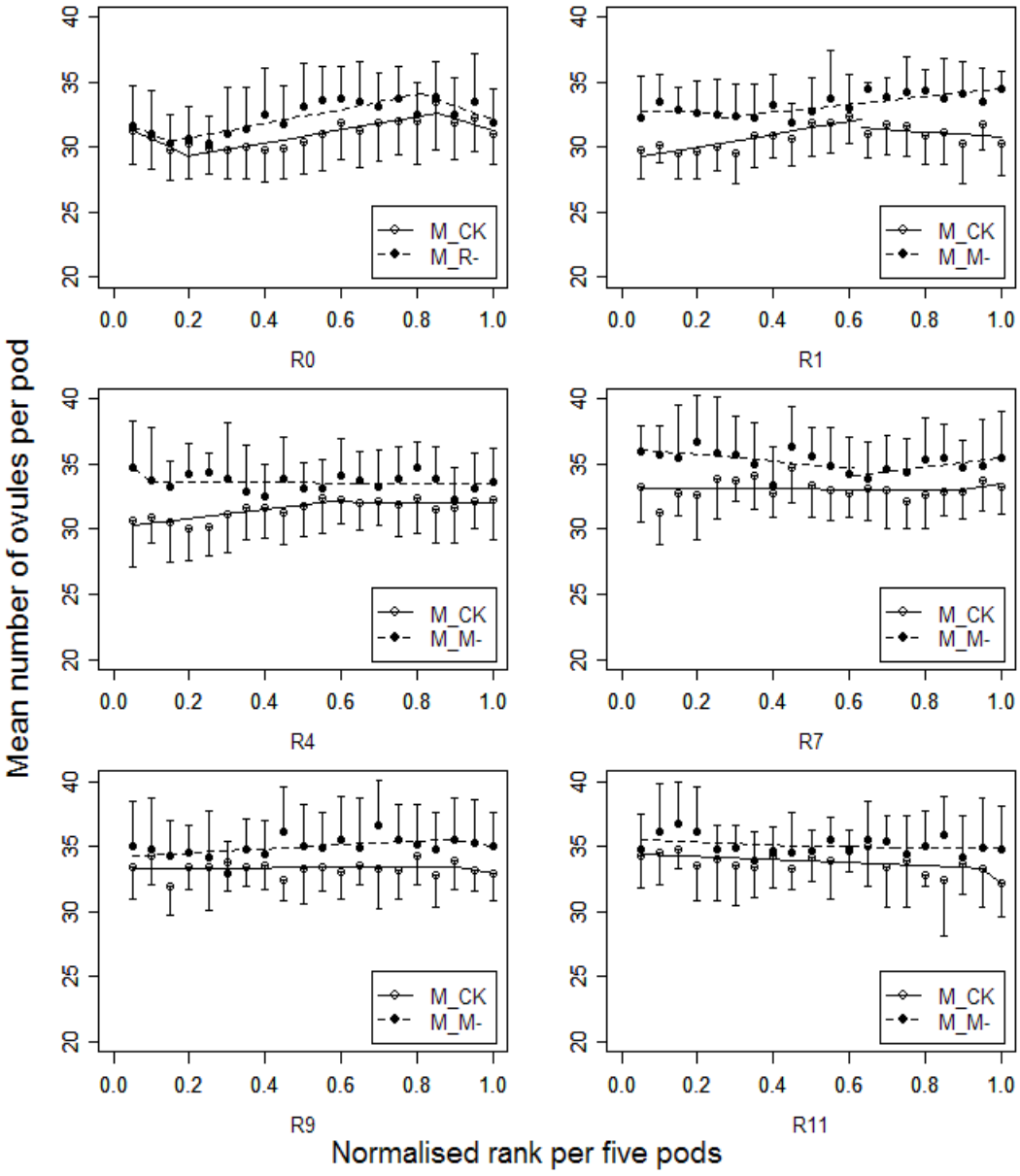


Figure 3.9: Mean number of ovules per pod along the inflorescence on the main stem R0 and the ramifications R1, R4, R7, R9 and R11 (Variety: Mendel). Dots and circles represent mean number of ovules per pod for the control (M_CK) and clipped (M_R-) plants respectively. Vertical bars represent standard deviations. Fit lines using segmented regression are shown.

Table 3.4: Effect of clipping main stem treatment (M_M-) on the number of ovules and seeds per pod on ramifications R0, R1, R4, R7, R9 and R11 compared to the clipped plants (M_CK). Values are mean ± SD. Values with different superscripts (within a column) differ significantly using the Tukey's HSD test at $P < 0.05$

	Mean total number of seeds per axis ± SD		Mean total number of pods per axis ± SD	
NO. Ramification	M_CK	M_M-	M_CK	M_M-
R0	30.9 ± 2.7^{a}		24.2 ± 6.8^{b}	
R1	30.7 ± 2.6^{a}	33.1 ± 2.4^{a}	22.4 ± 7.1^{a}	26.0 ± 7.6^{ab}
R4	31.6 ± 2.5^{b}	$33.7 \pm 2.7a$	21.4 ± 8.1^{a}	27.3 ± 6.7^{ab}
R7	33.1 ± 2.5^{bc}	35.2 ± 3.1^{b}	21.7 ± 9.2^{a}	24.3 ± 9.2^{b}
R9	33.4 ± 2.2^{bc}	35.0 ± 3.0^{b}	21.0 ± 9.8^{a}	23.8 ± 8.8^{bc}
R11	33.8 ± 2.7^{bc}	35.0 ± 2.7^{b}	24.4 ± 8.8^{b}	21.7 ± 9.0^{c}
df	5	4	5	4
F	10.7	11.7	89.8	23.9
P-value	$***$	$***$	$***$	$***$
M_CK vs. M_M-	$df = 1, F = 32.7, p < 0.001$		$df = 1, F = 672.7, p < 0.001$	

$***, P < 0.001$

The measurements of the control plants (Treatment M_CK) suggested that the mean number of ovules per pod increased from R0 to R11 ($30.8 - 33.8$) between the ramifications from top to bottom (Tukey's HSD comparison test, $P < 0.001, df = 5, F = 10.7$, Table 3.4, M_CK).

3.5.2 Effect of clipping the main stem or ramifications on the number of seeds per pod

Effect of pod position and clipping treatments on the main stem

Effect of pod ranks The segmented regression demonstrated that the number of seeds per pod remained constant before the 2nd breakpoint (0.77 ± 0.04, coefficient b_1 in Eqn. 2.2, $t = -1.2, P > 0.1$) and decreased significantly after that point with rank on the main stem in the control plants (coefficient b_1 in Eqn. (1), Fig. 3.10, R0, $t = -2.8, P < 0.05$). However, the number of seeds per pod did not show a significant difference before and after the 2nd breakpoint (0.6 ± 0.04, coefficient b_1 in Eqn. 2.2, $t = -0.5, P = 0.64$) with the pod rank in the clipped plants.

Effect of clipping ramifications Segmented regression indicated that no significant changes in trend (slope) existed before and after the 1st breakpoint on the main stem

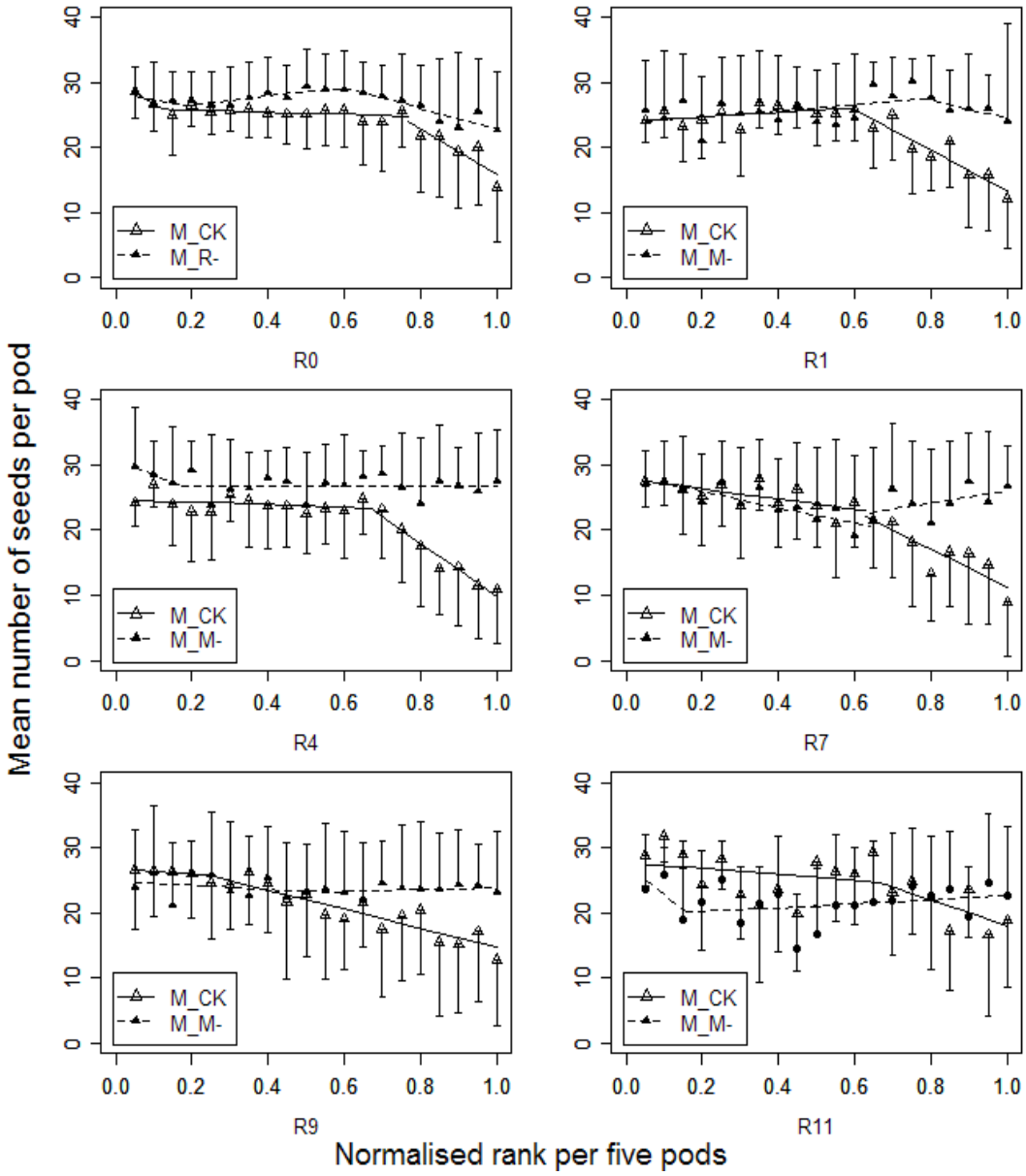


Figure 3.10: Mean number of seeds per pod along the inflorescence on the main stem R0 and the ramifications R1, R4, R7, R9 and R11 (Variety: Mendel). Dots and circles represent mean number of seeds per pod for the control (M_CK) and clipped (M_R-) plants, respectively. Vertical bars represent standard deviations. Fit lines using segmented regression are shown.

Table 3.5: Effect of clipping main stem treatment (M_M-) on the total number of pods per axis and seeds per axis on ramifications R0, R1, R4, R7, R9 and R11 for clipped plants and control plants. Values are mean ± SD. Values with different superscripts (within a column) differ significantly using the Tukey's HSD test at $P < 0.05$.

	Mean total number of seeds per axis ± SD		Mean total number of pods per axis ± SD	
NO. Ramification	M_CK	M_M-	M_CK	M_M-
R0	1062.3 ± 703^a		43.9 ± 11^a	
R1	475 ± 29^b	611 ± 65	21.2 ± 4.7^b	23.9 ± 5.3^b
R4	471 ± 42^b	720 ± 60	22.1 ± 6.5^b	26.7 ± 5.4^b
R7	543 ± 36^b	735 ± 66	25.1 ± 5.9^b	27.4 ± 6.2^b
R9	496 ± 49^b	713 ± 37	23.6 ± 8.1^b	30.0 ± 6.9^b
R11	633 ± 90^b	766 ± 128	26.0 ± 8.1^b	35.3 ± 8.7^c
Total number per plant	4912 ± 194	4927 ± 51	150 ± 2	144 ± 4
df	5	4	5	4
F	23.3	0.69	23.3	3.24
P-value	$***$	ns	$***$	$*$
M_CK vs. M_M-	$df = 1, F = 26.9, p < 0.001$		$df = 1, F = 16, p = 0.0001$	

$***, P < 0.001; **, P < 0.01; *, P < 0.05$; ns, not significant

in the control (coefficient b_2 in Eqn. (2), 0.12 ± 0.05, $t = 1.1, P = 0.29$) and clipped plants (0.18 ± 0.07, $t = 0.9, P = 0.39$). However, the changes in trend before and after the 2^{nd} breakpoint were significant in the control (coefficient b_3 in Eqn. 2.2, $t = -6.6, P < 0.001$) and clipped plants (coefficient b_3 in Eqn. 2.2, $t = -4.1, P < 0.05$). Therefore, the change in the number of seeds per pod in the distal pods was larger than in the basal pods. This result indicated that the effect of clipping ramifications on the number of seeds per pod depended on the position of pods within the main stem.

The number of seeds per pod did differ significantly before and after the 2^{nd} breakpoint (coefficient b_3 in Eqn. 2.3, $t = 3.8, P < 0.001$), but not for the 1st breakpoint (coefficient b_3 in Eqn. 2.2, $t = -0.2, P > 0.1$) between the control and clipped plants. Clipping ramifications did have a significant effect on the number of seeds per pod (Fig. 3.10, R0).

The mean numbers of seeds per axis were larger in the clipped plants (t-test, $t = 2.9, df = 386, P < 0.05$). An increase in the total number of seeds per axis was present, ranging from 1054 ± 282 (mean ± SD) to 1568 ± 398 (mean ± SD) in the control and clipped plants, respectively.

Effect of pod position and clipping treatments between ramifications

Effect of pod ranks The mean number of seeds per pod did not differ significantly in pods before breakpoints for each ramification on the control plants (coefficient b1 in Eqn. 2.1, breakpoint: R1: $0.6 \pm 0.04, t = -0.8, P = 0.44$; R4: $0.67 \pm 0.03, t = -1.9, P = 0.08$; R7: $0.63 \pm 0.08, t = -0.9, P = 0.4$; R9: $0.24 \pm 0.17, t = -1.1, P = 0.2$ and R11: $0.66 \pm 0.2, t = -1.7, P = 0.12$). However, significant decreases were present in the number of seeds per pod with rank after the breakpoints for each ramification in the control plants (coefficient b_2 in Eqn. 2.1, $P < 0.001$ for each ramification). The number of seeds per pod did not vary significantly with the pod rank in the clipped plants (coefficient b_2 in Eqn. 2.1, $P > 0.1$ for each ramification). The number of seeds per pod tended to decline with higher pod rank, but the decline was more severe along the inflorescence for ramifications R1, R4 and R7 (Fig. 3, coefficient b_2 in Eqn. (1), $P < 0.05$ for each ramification). Furthermore, as shown in Fig. 3, a large variability was present in the number of seeds per pod along the inflorescence on ramification R11.

Effect of clipping the main stem Clipping the main stem did not significantly influence the number of seeds per pod before breakpoints on ramifications R1, R7, R9 and R11 (coefficient b_3 in Eqn. 2.3, $P > 0.1$ for each ramification), but it had an impact on the ramification R4 (coefficient b_3 in Eqn. 2.3, $t = 3.6, P = 0.001$). The differences in the number of seeds per pod were significant between the control and clipped plants after breakpoints for ramifications R1, R4, R7 and R9 (coefficient b_3 in Eqn. 2.3, $P < 0.001$ for each ramification). Thus, the results indicated that clipping the main stem had a greater influence on the number of seeds per pod on the upper ramifications than on the lower ramifications.
The mean number of seeds per pod on the main stem and on ramification R11 was higher than on ramifications R1, R4, R7 and R9 in the control plants. However, the mean number of seeds per pod decreased with ramifications from top to bottom in the clipped plants (Table 3.4). The mean total number of seeds per ramification increased ($F = 26.9, df = 1, P < 0.005$, ANOVA, Table 3.5) in the clipped plants compared to the control plants. However, the total number of seeds per plant was not different (Mean $\pm$ SD, M_CK: 4912 ± 194 and M_M-: 4927 ± 51, t-test, $t = -0.72, df = 9, P = 0.5$).

3.5.3 Effect of clipping the main stem or ramifications on the number of pods

Effect of pod ranks

The number of pods per inflorescence depends on the number of developed pods and aborted pods, which can be described by the ratio of the number of aborted pods to the total number of pods at each pod rank (Pod abortion). This ratio was large at

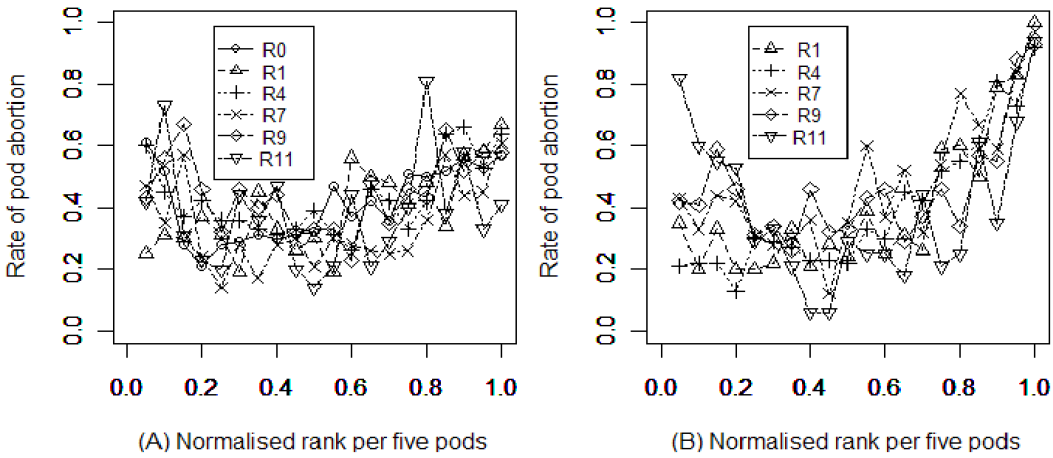


Figure 3.11: Rate of pod abortion according to the normalised rank on inflorescences R0, R1, R4, R7, R9 and R11 (Variety: Mendel).

the basal position, then remained constant and increased with the pod rank along the inflorescence (Fig. 3.11) in the control (M_CK) and clipped plants (M_M-).

Effect of clipping treatment The rate of pod abortion was not significantly different between inflorescences in the control ($F = 1.65, df = 5, P = 0.15$) and clipped plants ($F = 0.6, df = 4, P = 0.66$) (Fig. 3.11). Furthermore, the pod abortion rate did not differ significantly between the control and clipped plants ($F = 1.2, df = 1, P = 0.28$). All pods aborted at the end of inflorescence on the clipped plants.
Ramification clipping induced a significant increase in the number of pods on the main stem (t-test, $t = -3.1, df = 17, P < 0.01$), with an average of 58 ± 13 (mean $\pm$ SD) pods in the plants with clipped ramifications compared to 44 ± 11 (mean $\pm$ SD) pods in the control plants.
Furthermore, the mean total number of pods per axis also increased compared to the control plants (ANOVA, $F = 16, df = 1, P < 0.0001$, Table 3.4). The plants with clipped main stems had an average increase of 5 pods for each ramification compared to the control plants. However, the mean total number of pods per plant was not significantly different (t-test, $t = -0.12, d = 8, P = 0.3$) between the control (M_CK: 144 ± 4 (mean $\pm$ SD); R0, R1, R4, R7 and R11) and clipped plants (M_M-: 150 ± 2 (mean $\pm$ SD); R1, R4, R7 and R11, Table 3.5).
The mean total number of pods per axis was larger on the main stem than on ramifications and did not differ significantly among ramifications (TukeyHSD's test, $P < 0.01$, Table 3.5). In addition, ramification clipping induced an increase of 30% of the mean seed weight (data not shown) on the main stem.

3.5.4 Effect of clipping the ramifications and 20 basal flowers on the number of ovules per ovary, seeds per pod and mean seed weight

The number of ovules and seeds per pod in the clipped plants did not differ significantly between the clipped and control plants for the Pollen variety (Treatment P_R-') (ANOVA, $P > 0.1$), as shown in the fig. 3.12. The result was consistent with the study of [Bell, 1985], the removal of flowers did not influence the number of seeds per pod. However, the mean seed weight for the clipped plants increased. Clipping of all the ramification and basal flowers induced an increase of 30% of the mean seed weight on the main stem. The results indicated that the more basal pods deplete or intercept the assimilate to the detriment of the more distal pods within an inflorescence.

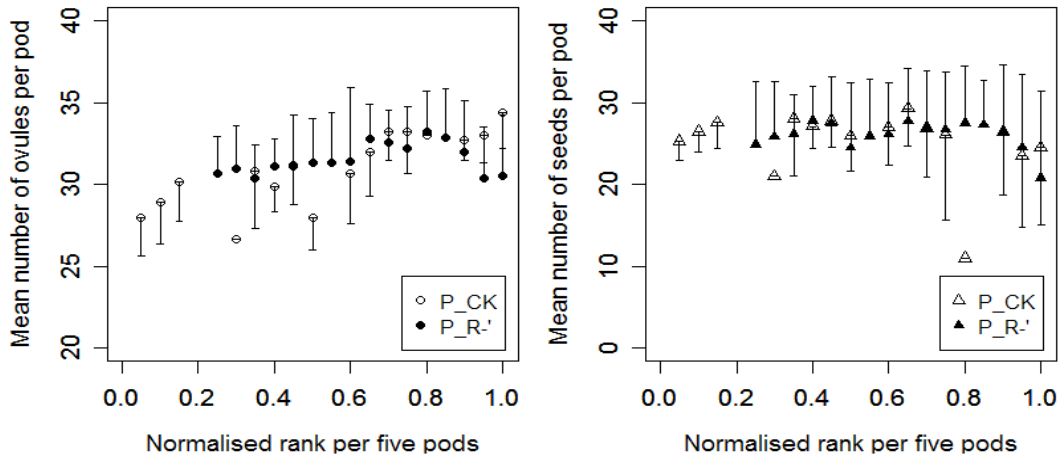


Figure 3.12: Number of ovules and seeds per pod for the clipped and control plants (Variety: Pollen).

3.6 Difference of the number of ovules and seeds per pod between varieties

3.6.1 Number of ovules and seeds per pod on each inflorescence

For each inflorescences, the number of ovules per pod is Exocet > Gamin > Pollen > Mendel (Fig. 3.13A). However, there is no significant difference between the varieties Gamin, Pollen and Mendel. In contrast, the variation of the number of seeds per pod is

quite obvious. The number of seeds per pod for Gamin variety is obviously small even if it has larger number of ovules per pod. There is no big difference between the other varieties for the number of seeds per pod (Fig. 3.13B).

3.6.2 Number of ovules and seeds per pod between inflorescences for each variety

The number of ovules per pod increased with ramifications from top to bottom for the variety Exocet, Pollen and Mendel. However, for the Gamin variety, the number of ovules per pod did not show the tendency of increase with ramifications (Fig. 3.14A). The number of seeds per pod did not differ significationly with ramifications for the variety Pollen and Mendel. However, the number increased with ramifications from top to bottom for the Exocet variety. There was same tendency of the number of seeds per pod for the Gamin variety between ramifications, but the ramification R11 had small number of seeds per pod for this variety (Fig. 3.14B).

3.6.3 Comparison of seed yield

The mean seed weight for the four varieties are presented in fig. 3.15. The Gamin variety had higher mean seed weight than the others ($P < 0.001$, ANOVA).

3.7 Conclusion

In the thesis, WOSR plants varied in both intra-inflorescence and inter-inflorescence yield components. Pod position and time of pod appearance, related to assimilate availability, had effects on yield components in varying degrees (Table 3.6). In addition, the number of ovules and seeds per pod did differ between varieties and between inflorescences for one variety.
[Allen and Morgan, 1975] compared the development and yield of four varieties in oilseed rape. They found that the number of seeds per pod and pods per axis were positively correlated to the index of leaf area [Gabrielle et al., 1998]. The results indicated that the assimilate availability during the flowering period is an important factor to determine the seed yield. The variation between varieties may be due to the difference of development time [Allen and Morgan, 1975; Mendham et al., 1981a].

3.7.1 Effect of pod position

Pod position (within one inflorescence and between inflorescences in a plant) plays an important role in the number of ovules per pod, seeds per pod and pods per axis [Brookes et al., 2010; Ortiz et al., 2009]. The number of ovules per pod differed between

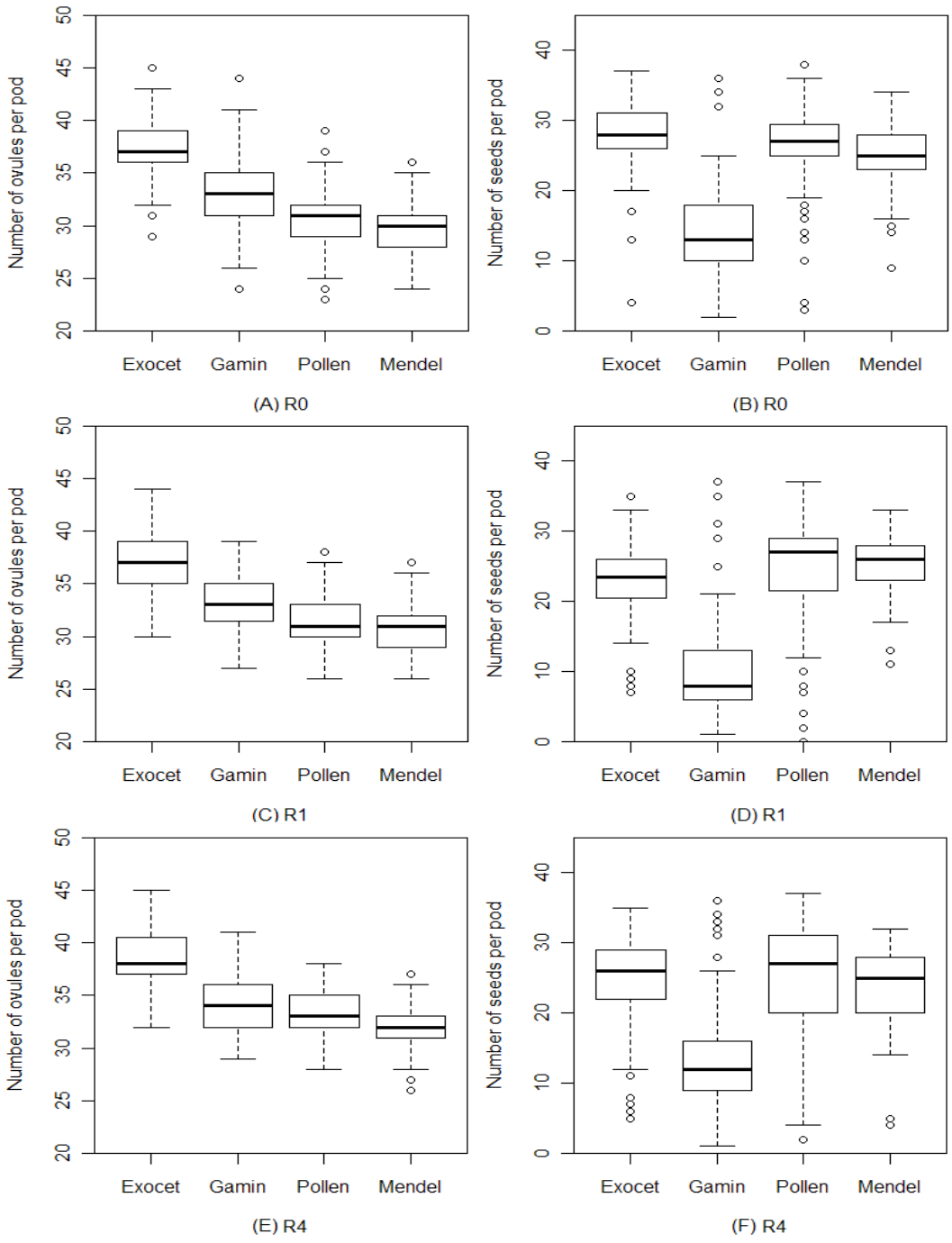


Figure 3.13: Boxplot of the number of ovules per pod (A) and seeds per pod (B) for the four varieties on the inflorescences R0, R1, R4, R7, R9 and R11.

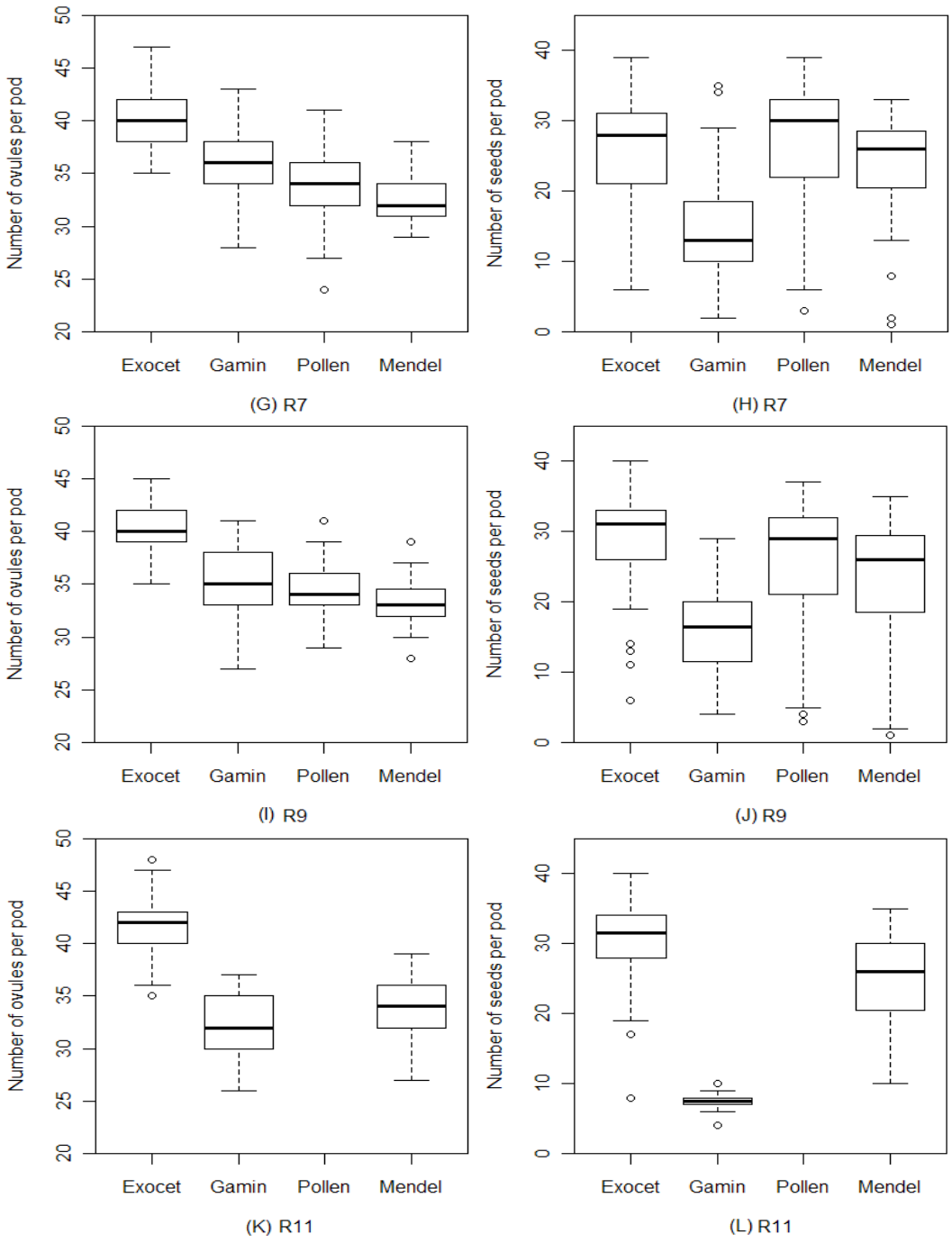


Figure 3.13: Boxplot of the number of ovules per pod (A) and seeds per pod (B) for the four varieties on the ramifications R0, R1, R4, R7, R9 and R11. (Continued..)

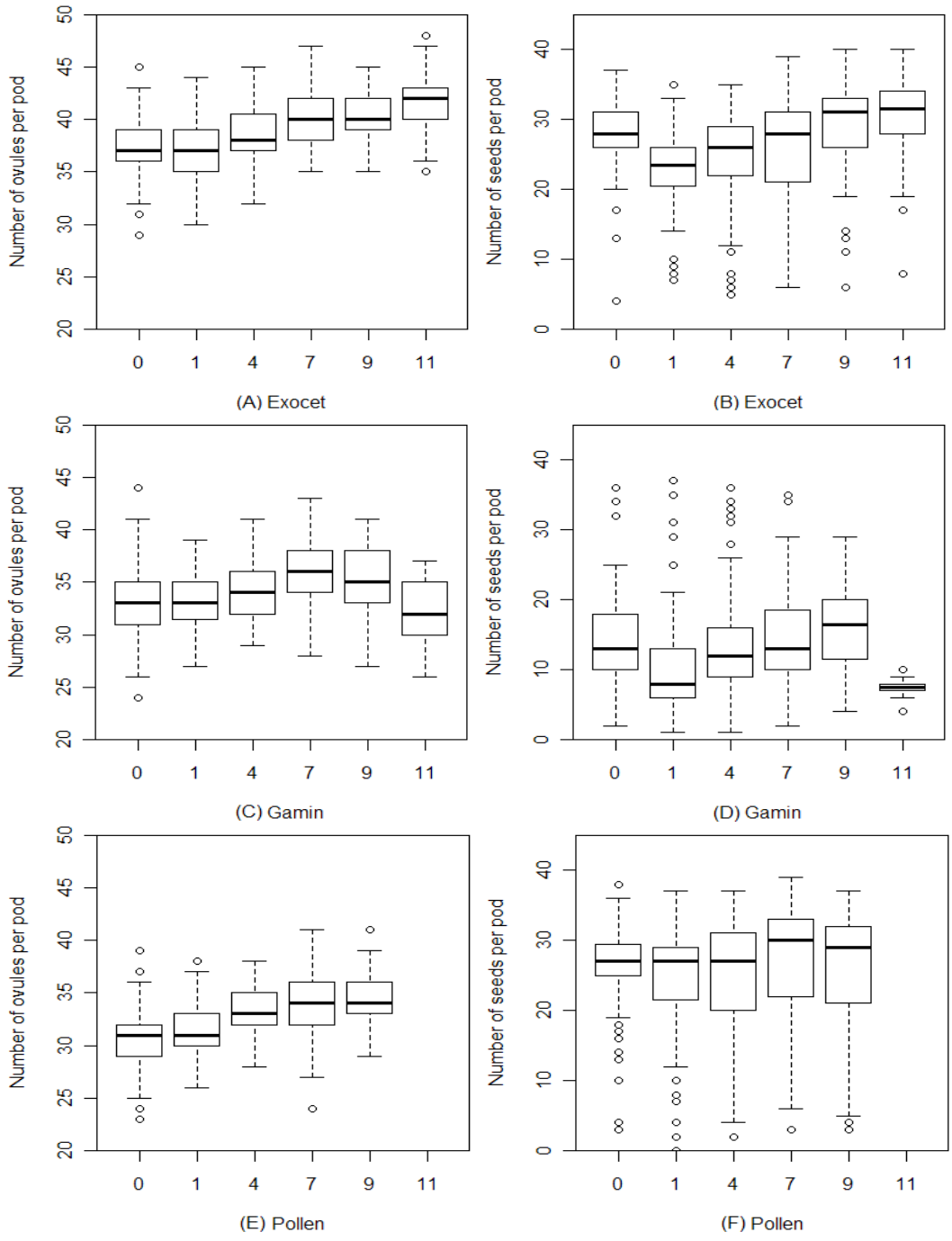


Figure 3.14: Boxplot of the number of ovules per pod (A) and seeds per pod (B) on the ramifications R0, R1, R4, R7, R9 and R11 for each variety.

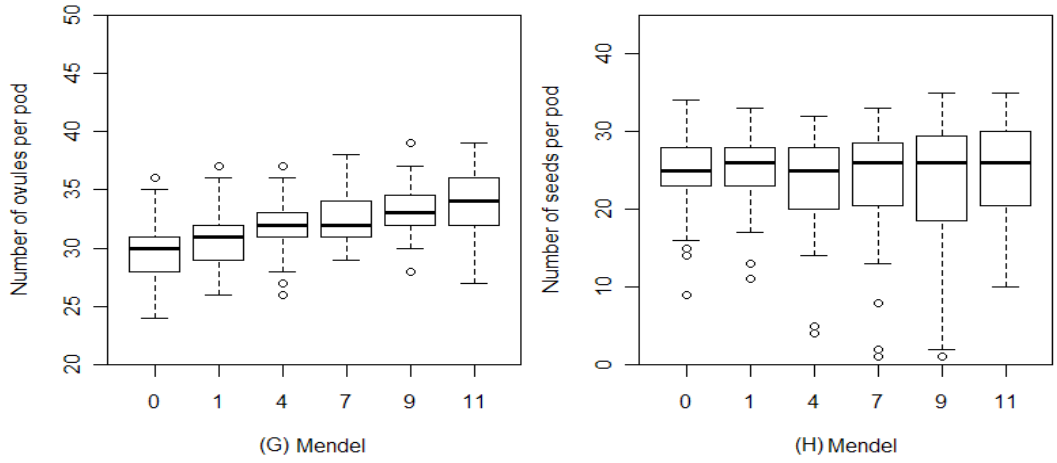


Figure 3.14: Boxplot of the number of ovules per pod (A) and seeds per pod (B) on the ramifications R0, R1, R4, R7, R9 and R11 for each variety. (Continued..)

two axes. On the main stem, the number of ovules per pod was large at the basal positions followed by small numbers, then increased along the inflorescence but decreased at the distal positions. Furthermore, the number of ovules per pod was small at the beginning of ramifications R1 and R4. This difference could be due to the complex developmental patterns of inflorescences in WOSR. Ramifications are initiated from the bottom to the top, however, the expansion of ramifications occurs in the inverse order of their initiation and is delayed compared to the main stem. The duration between initiation and expansion is longer for basal ramifications than for upper ramifications. As a result, initiated pods on the basal ramifications have a longer developmental period, which could explain the greater number of ovules per pod in the lower ramifications.
The pod rank appeared to be the major determinant of the number of seeds per pod within one inflorescence. The decreasing pattern observed could be due to a limited access to assimilate because they have been depleted or intercepted by more proximal pods along the stem [Brookes et al., 2010; Lee, 1988; Stephenson, 1981]. This result indicates the importance of the pod position because the farther the pod is from the leaves, the smaller its number of seeds [Pate and Farrington, 1981]. The interception of assimilates by proximal pods could explain why the number of seeds per pod in distal pods did not vary with the pod rank in the clipped plants, as the competition for assimilates is assumed to be lower for these plants.
The distribution of the number of seeds in the plant architecture is more complex. The main stem had larger number of seeds per pod than the ramifications. These results might be due to the apical dominance effects [Ruiz de Clavijo, 1995]. Apical dominance is an inhibitory influence exerted by the main stem on the development of axillary inflorescences and is best demonstrated via main stem removal [Cline, 1997]. If the main

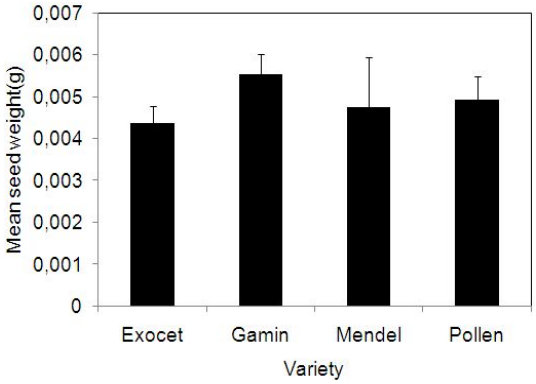


Figure 3.15: Mean seed weight on the main stem for the four cultivars. The short lines represent the error bounds.

stem is clipped, then apical dominance is released and one or more of these lower lateral inflorescences begins to grow out. This phenomenon can explain why the main stem had more pods than the ramifications and why the main stem had a slower decrease in the number of seeds per pod according to the rank than the ramifications. Furthermore, the main stem flowers earlier and has a competitive advantage over the ramifications as the supply of assimilates is higher during main stem growth [Pate and Farrington, 1981]. Also, at the end of the flowering period, the competition for assimilates increased as leaf area decreased, the number of pods increased and the pod canopy created deep shade [Mendham et al., 1981a].
When clipping the main stem or ramifications, the demand for assimilate and thus the trophic pressure in the entire plant decreases. Plants subjected to clipping treatments developed more pods and more seeds per pod than control plants that were not subjected to clippings. These results are similar to other cases in which fruit production in late opening flowers has been increased experimentally by removing early opening flowers or stigmas [Ehrlen, 1993; Lehtila and Syrjanen, 1995]. [Hiei and Ohara, 2002] indicated that main stem clipping enhances the performance of lateral branches in Melampyrum japonicum, as more ovules and more seeds per pod as well as more pods in ramifications were obtained. The clipping treatments induced significant variations in the number of ovules, seeds and pods in the plants. The comparison of the two varieties highlighted different behaviors. For the Mendel variety, the number of ovules increased in pods that emerged immediately after the clipping, regardless of which axis was clipped (main stem: M_M- or ramifications: M_R-). Ramification clippings were performed approximately in the rank 20th on the main stem, and the number of ovules and seeds per pod increased from normalised rank 0.2 compared to the control plants. This variety appears to have a quick response to the loss of organs, resulting in the fast production of new reproductive organs [Wright and Meagher, 2003]. Furthermore, the

Table 3.6: Variation of yield components of WOSR with different factors

Factors	Number of ovules per pod	Number of seeds per pod	Number of pods per axis
Pod rank	a	b	NA
Ramification position	+	+	ns
Clipping ramifications (M_R-)	+	+	+
Clipping main stem (M_M-)	+	+	+
Clipping basal flowers (P_R-')	ns	ns	+
Time of pod appearance	ns	c	ND

'a' represents first decrease, then increase and decrease again. 'b' represents first remain constant, and then decrease. 'c' represents the time of pod appearance had effect on the number of seeds per pod. '+' and '-' represent 'increase' or 'decrease' with the factors, respectively. 'ns' represents not significant. 'NA' not appropriate. 'ND' no data to analyse

number of pods significantly increased on the main stem in the clipped plants. For the clipping the main stem plants, the number of ovules per pod in ramifications R1 and R4 did not vary with the pod rank. In addition, the number of ovules per pod was also larger in all of the ramifications in the clipped plants compared to the control plants. The number of seeds per pod did not decrease with higher pod rank on the main stem and ramifications in the clipped plants. However, the total number of seeds and pods per axis did not show any significant difference between the control and clipped plants. The results indicated that WOSR has the potential for growth after flowering, which compensates for losses of flowers, pods and branches [Diepenbrock, 2000; Wright and Meagher, 2003]. When clipping the ramifications or flowers, the Mendel variety can produce the new reproductive organs to compensate the losses. Under normal conditions, the plants can not reach the potential of production due to the supply of assimilates. The Pollen variety was subjected to a more severe clipping than Mendel, all of the ramifications and basal 20 flowers were removed, thus the supply of assimlilates may decrease correspondingly, and yet, the number of ovules and seeds per pod remained unchanged between control and clipped plants. However, a compensation of the loss of reproductive organs was observed by an increase in mean seed weight and in the number of pods per inflorescence. Furthermore, a large variability on the ramification R11 in the control and clipped plants was present, which could be due to assimilate availability. Most of the pods in the plant stopped growing, so the competition for assimilates should be smaller at the end of reproductive stage. However, the supply of assimilates in this period also decrease. More studies need to be done to analyze the cause of variation in the ramification R11.

3.7.2 Effect of time of pod appearance

The data analysis reveals that the time of pod appearance had impact on the number of ovules and seeds on the whole plant level. The number of ovules per pod was small at the beginning of flowering period, then the number increased and remained constant with the time of pod appearance. The number of pods with small number of seeds increased at the end of flowering period. In addition, the number of aborted ovules was large at the beginning, then decreased and remained constant, but increased with the time of pod appearance. The phenomena could be due to the developmental pattern of WOSR. Pods develop acropetally within one inflorescence. Thus, the early developed pods have a competitive advantage to obtain assimilates over later formed pods. Flowering on the later developing secondary inflorescences may continue for some time after the main stem has finished flowering. Older pods at the base of these flowering inflorescences are well developed, while new flowers are still being initiated at the tips. Thus, the number of seeds that develop in these pods will be influenced by resource availability.
This pattern of ovule abortion is correlated inversely with the number of flowers in the fields. Few flowers open at the beginning of the flowering and only on the main stem. The number of ovule abortions progressively increases while flowers appear on all of the ramifications. Finally, most flowers become pods and inflorescences gradually stop growing, which results in a lower number of flowers in the field at the end of the reproductive period and, hence, a reduced amount of pollen grains for late flowers. This reduced pollen count corresponds to the variation of pollen quantity and quality during the flowering period, for example, the inefficient pollinator [Berjano et al., 2006], and thus leads to different pollination conditions, which can affect negatively the fertilization process and the abortion of seeds [Brookes et al., 2010; Brunet and Charlesworth, 1995]. Furthermore, the variation of aborted ovules could be a cause of the variation of the rate of pod abortion with the pod rank. Because the survival of pods depends on the number of seeds per pod [Ganeshaiah et al., 1986]. Plant architecture could also induce differences in the ability of a flower to be pollinated. The density of pollen might vary at different locations in the WOSR canopy [McCartney and Lacey, 1991].
Furthermore, a correlation exists between the position of a pod and its date of emergence in the plant, but these two factors are difficult to differentiate. However, we know that the ratio of supply of assimilates to demand decreases with time during the reproductive period [Jullien et al., 2010]. Furthermore, the number of seeds per pod was smaller for the latest developed pods, which is in accordance with the hypothesis that resource availability is an important factor in ovule abortion [Bouttier and Morgan, 1992a]. In conclusion, our results indicate that in WOSR, the amount of available assimilates was the primary determinant of pod and seed production during the period of flowering and pod setting. The distribution of resources was significantly affected both by the position of a pod within inflorescences, and by the position of the inflorescences within a plant. Basally positioned pods had a distinct advantage in acquiring resources due

to their greater proximity and earlier development time. Increases in pod rank and ramification position affect appearing time, which can be observed through the change in assimilate availability on the entire plant.
Our study focuses primarily on the effect of assimilate availability on yield components. However, pollination also influences the yield. To study the impact of the pollination of the yield components, a probabilistic model has been developed to simulate the distributions of the number of ovules and seeds per pod. The model can also be used to simulate the distribution of the number of pollen grains [Wang et al., 2009]. This model allows us to estimate the distribution parameters of the number of pollen grains per stigma and discuss the effect of pollination deficit on the number of seeds per pod.

Part II

MODELIZATION

Chapter 4

Modelization - model description, calibration and parameter identification

4.1 Model description

Seed production involves several floral components, including steman, pistil and ovules (Fig. 4.1). Seed production depends on the successful completion of pollination and fertilization [Gillaspy et al., 1993]. The compatible pollen has to germinate on the stigma of the pistil, and forms a pollen tube. This pollen tube then grows through the style and the ovular micropyle to deliver two sperm cells in the embryo sac. There a double fertilization occurs; one of the two sperm cells fertilizes the ovule, while the other fuses with two haploid polar nuclei in the central cell (Fig. 1.1). The fertilized ovules develop into seeds and the ovary enlarges and becomes a fruit. The association of ovule and pollen is likely to create a seed but in some conditions there may be an abortion of the seed due to a fertility problem [Arathi et al., 1999]. We simulated the processes of seed production using several probabilistic distributions and simplied the processes. The model mainly focuss on the stages with higher probabilities of abortions. Based on the biological description, theoretically, the model can reproduces the main steps from flower appearance to seed production with the five following probability distributions.

(1) Distribution of the number of ovules in the ovary

(2) Distribution of the number of pollen grains landing on a flower;

(3) Distribution of the number of fertilized ovules;

(4) Viability of seeds: the probability for a fertilized ovule to develop into a mature seed;

(5) Abortion of a pod according to its number of seeds.

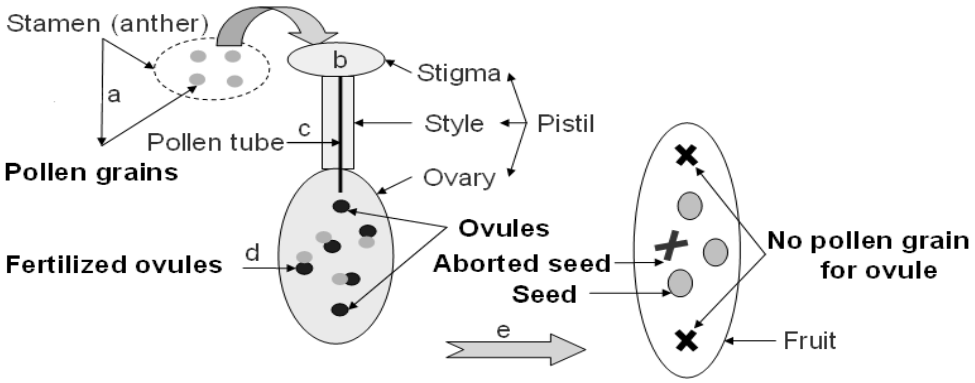


Figure 4.1: Schematic presentation of the events of seed development. Seed production involves several processes and floral components: pollen production by the anther, deposition of pollen grains (a) on the stigma (b), pollen germination and growth of the pollen tube (c), fertilization (d) and development of embryo and seed (e).

Firstly, Y denotes the random variable of the number of ovules in the ovary. At first, the number of ovules per flower is different between species, even for the same species, the number of ovules per flower is also uncertainty. Binomial distribution with parameters N and b is used to describe the number of ovules per ovary. N is seen here as the maximum number of ovules and b is the probability of survival of an ovule. Thus the probability that an ovary contained y viable ovules is given by the equation 4.1:

$$P(Y = y) = C_N^y b^y (1-b)^{N-y} \tag{4.1}$$

However, thanks to the variation of the number of ovules for the measurements, N varied a lot when we estimated the parameters b and N using different data set. If we fit the N to estimate the b, it will influence the the following estimations. Fortunerately, we found that the mean number of ovules per pod remained stable for different data set. In addition, the number of ovules per flower tends to cluster around the mean. Therefore, the distribution of the number of ovules per ovary is described with a normal distribution with mean μ and standard deviation σ. Thus the probability that an ovary contained y viable ovules is given by the equation 4.2.

$$f_Y(y) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \tag{4.2}$$

Secondly, the number of pollen grains that arrive into the stigma of a flower is described by the random variable T. According to the assumption of [Falque et al., 1995], pollen tubes reach ovules in a similar way whether or not these ovules have already been reached by another pollen tube. And [Sakai and Kojima, 2009] also found that seed

production does not depend on the time of ovule fertilized within a flower. To model the complex phenomenon of pollination, three probability distributions have been compared: Pareto law, negative binomial law and lognormal law.

- Pareto law is defined by the probability density function 4.3, with parameters a, xo:
$$f_P(t) = \frac{ax_O}{t^{a+1}} \tag{4.3}$$

- The negative binomial distribution is defined by the probability 4.4:
$$f_N(t) = C_{t+K-1}^{t} \cdot a^K \cdot (1-a)^t \tag{4.4}$$
where K, a are the probability parameters.

- The log-normal distribution is defined by the probability density 4.5:
$$f_L(t) = \frac{e^{-\frac{(ln^t - m)^2}{2s^2}}}{ts\sqrt{2\pi}} \tag{4.5}$$
where m, s are the parameters of the distribution.

As continuous density functions were chosen for discrete variables (number of pollen grains, number of ovules), these continuous distributions were corrected using the continuity correction [Pirie and Hamdan, 1972]. The discrete form can be obtained with the following relationship 4.6:

$$P(T = t) = f(t + 0.5) - f(t - 0.5) \tag{4.6}$$

The estimation results indicated that lognormal distribution can give the best result, thus we chose log-normal distribution in our model. This will be introduced in the section 4.3.1.
A pollen grain is assumed to be effective if it germinates and penetrates into the ovary to pollinate an ovule. The number of effective pollen grains (X) that can germinate and produce the pollen tube on a stigma is described by the random variable $X = kT$, k is the ratio of effective to total pollen grains. Thus, the number of effective pollen grains can be denoted by equation 4.7:

$$f(x) = \frac{1}{\frac{x}{k}\sigma\sqrt{2\pi}} e^{\frac{-(ln\frac{x}{k} - \mu)^2}{2\sigma^2}} \frac{1}{k} = \frac{1}{x\sigma\sqrt{2\pi}} e^{\frac{-[ln^x - (ln^k + \mu)]^2}{2\sigma^2}} \tag{4.7}$$

This function is also a log-normal distribution.
During the general parameterization of model parameters by optimization, only one

parameter s is optimized to define log-normal distribution, and the another parameter m is subsequently derived from Ao by iteration using the constraint 4.8

$$Ao = \frac{m}{m + \frac{s^2}{2}} \tag{4.8}$$

Ao is an empirical coefficient that ranges from 0 to 1, thus, we can compute the effective number of pollen grains using the following equation 4.9:

$$f(t) = \frac{1}{x\sigma\sqrt{2\pi}} e^{\frac{-[ln^x - (ln^k + \frac{Ao \cdot s^2}{2(1-Ao)})]^2}{2\sigma^2}} \tag{4.9}$$

One effective pollen grain is necessary and sufficient for the fertilization of one ovule. Z denotes the number of fertilized ovules and we have the equation $Z = min(X, Y)$. The probability to get y fertilized ovules is given by the equation 4.10:

$$\begin{aligned} P(Z = y) &= P(X = y)P(Y > y) \\ &+ P(Y = y)P(X \geq y) \end{aligned} \tag{4.10}$$

Then, the fertilized ovules may develop into mature seeds with probability p because of seed viability. This viability is supposed to be linked to several factors, and mainly the resource competition. A fertilized ovule is more likely not to form a seed when there is a high competition for resources. If S is the number of fertilized ovules to form seeds, the probability to get i seeds is given by the equation 4.11:

$$\begin{aligned} P(S = i) = &\sum_{y=0}^{N} C_y^i p^i (1-p)^{y-i} P(Y = y) P(X \geq y) \\ &+ \sum_{y=0}^{N} \sum_{k=i}^{y-1} C_k^i p^i (1-p)^{k-i} P(X = k) P(Y = y) \end{aligned} \tag{4.11}$$

The demonstration of equation 4.11 is given in appendix A.
Lastly, if a pod contained too few seeds, then it may abort. In the study of frequency distribution of the number of seeds in pod of *Leucaena leucocephala* (Lam), Ganeshaiah found that the formation of pods was related to the number of seeds in it [Ganeshaiah et al., 1986]. This relationship was modelled with the beta function in the model, which is a flexible function commonly used in biological models, see [Yin et al., 2003] for an example. The probability of pod survival is defined by a function $F(i)$ that depends on the number i of seeds per pod contained inside with parameters α, β.

$$F(i) = \sum_{j=1}^{i} \frac{g(j)}{Mo} \tag{4.12}$$

$$g(j) = \frac{1}{N}\left(\frac{j-0.5}{N}\right)^{\alpha-1}\left(1-\frac{j+0.5}{N}\right)^{\beta-1}$$

$$Mo = \sum_{j=1}^{N} g(j)$$

In Equation 4.13, N is the maximum number of ovules per ovary, $F(i)$ is a normalized function that describes the cumulative probability of pod survival.
During the general parameterization of model parameters by optimization, only one parameter (Bo) is optimized to define the beta function, and β is derived from Bo by iteration using the constraint 4.13:

$$Bo = \frac{\alpha - 1}{\alpha + \beta - 2} \tag{4.13}$$

Thus the $g(j)$ can be computed using the equation 4.14:

$$g(j) = \frac{1}{N}\left(\frac{j-0.5}{N}\right)^{\alpha-1}\left(1-\frac{j+0.5}{N}\right)^{\frac{\alpha-1}{Bo}-\alpha+1} \tag{4.14}$$

B is the random variable of the final number of seeds per pod, and its probability distribution is given by the following equation 4.15:

$$P(B = i) = P(S = i)F(i) \tag{4.15}$$

By combining these laws, we can compute the final number of seeds per pod. The model has been developed in Scilab 4.0 (INRIA-ENPC, 2006).

4.2 Model parameters

The model parameters were estimated according to the experimental data. Table 4.1 summarized the parameters of the model. Firstly, the parameters μ and σ were estimated for the normal distribution with maximum likelihood estimation method (MLE). Then their values were set to the estimated values and the parameters for pollen grain distribution, the probability of seed viability and the probability of pod abortion were estimated simultaneously with the Generalized Least Square Method (GLSQR) [Zhan et al., 2003].

4.3 Model calibration

The experimental data of different measurements and treatments were used to estimate the parameter values. The measurements of 2007-2008 and 2008-2009 on the main stem

Table 4.1: Model parameters

Parameters	Distributions	Description
μ	Normal	Parameter for the distribution of ovule number
σ	Normal	Parameter for the distribution of ovule number
s	Log-normal	Parameter for the distribution of pollen number
k	Log-normal	Parameter for the percentage of effective pollen number
Ao	Log-normal	Parameter for the distribution of pollen number (fix to 0.9)
p	Bernoulli	Parameter for the viability of seeds
α	Beta	Parameter for pod abortion (fix to 3)
Bo	Beta	Parameter for pod abortion

m and β can be calculated according to the constraint formula 4.8 and 4.13, respectively.

was used to analyze the difference of parameter values between two years. The data of clipping all the ramifications or 20 basal flowers were used to analyze the difference of different situation of assimilate supply. Furthermore, the difference of parameter values between pod position (within the main stem and between inflorescences) were analyzed.
Pearson product-moment correlation coefficient (R^2) is used to test the correlation between the observed values and the estimated values. It can be expressed as:

$$R = \frac{\sum (X - \mu_X)(Y - \mu_Y)}{N\sigma_X\sigma_Y} \tag{4.16}$$

where μ_X, μ_Y are the mean of X, Y variables; σ_X, σ_Y are the variance of X, Y variables; N is the sample size.

4.3.1 Selection of the distribution of the number of pollen grains per stigma

As mentioned above, the pollination of flower is a complex phenomenon, three distribution function (Pareto distribution, negative binomial distribution and lognormal distribution) were compared to see which one is better to describe it in our study. The experimental data in the section 2.3 was used to estimate the parameter values of model. Akaike information criterion (AIC) Akaike [1973] was used to choose the appropriate distribution of number of pollen grains. It is a tool for model selection by measuring the goodness of fit of a model that takes into account the number of parameters in the model and the number of observations.
The AIC is computed using the equation

$$AIC = 2k + n\left[ln(\frac{2\pi Rss}{n}) + 1\right] \tag{4.17}$$

where k is the number of parameters in the statistical model, n is the number of observations and Rss is the residual sum of squares. Rss can be computed

$$Rss = \sum_{i=1}^{n} [y_i - f(x_i)]^2 \tag{4.18}$$

The distributions of the number of pollen grains (Fig. 4.2C) and the probability of pod abortion (Fig. 4.2D) differed significantly between the three distributions. Table 4.2 presents the corresponding parameter values of model for each process. The distributions of observed and computed the numbers of ovules and seeds are presented in Fig. 4.2. The number of seeds per pod was better fitted with lognormal law for pollen grain number, as confirmed by the AIC values (Table 4.2). Thus, we choose the lognormal distribution to estimate the parameter value in the following estimations.

Table 4.2: Estimated parameters of the model for three distributions of number of pollen grains.

			Pollination distribution		
Description	Distribution	Parameter	Pareto	negative	lognormal
Distribution of ovule number	normal	μ	31.3	31.3	31.3
Distribution of ovule number	normal	σ	4.1	4.1	4.1
Distribution of pollen number	Pareto	xo	0.5*		
Distribution of pollen number	Pareto	a	0.095		
Distribution of pollen number	negative binomial	K		4*	
Distribution of pollen number	negative binomial	a		0.105	
Distribution of pollen number	log-normal	m			3.403
Distribution of pollen number	log-normal	s			0.105
Viability of seeds	Bernoulli	p	0.814	0.87	0.883
Pod abortion	Beta	α	3	3	3
Pod abortion	Beta	Bo	0.088	0.234	0.258
AIC			7416	6288	6285

'*' represents parameter not estimated.

4.3.2 Difference of estimated parameter values between years

The model parameters were estimated to analyze the difference between the two experimental years in 2007-2008 and 2008-2009. Fig. 4.3 shows the distrubution of the number of ovules (A) and seeds (B) per pod. There are more large number of ovules per pod in 2009 compared to 2008, but the mean number of ovules per ovary (μ) was same. The mean number of seeds per pod was larger in 2009 than 2008. The distribution of the number of pollen grains (s) was slightly large in 2009 (Fig. 4.3C) and the percentage

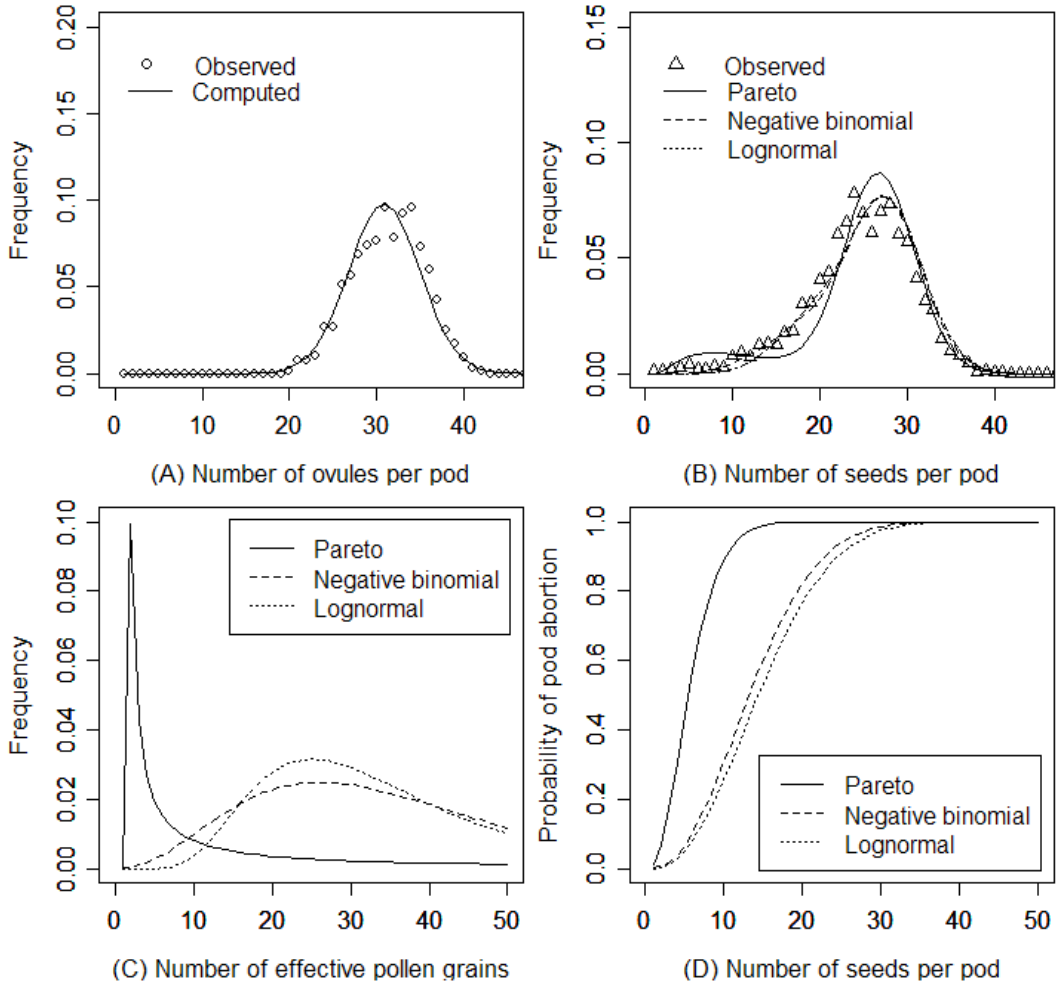


Figure 4.2: (A) Distribution of number of ovules per pod for the data in 2008. (B) Distribution of number of seeds per pod for three distributions Pareto, negative binomial and log-normal. (C) Distribution of number of effective pollen grains for three distributions; (D) Probability of pod abortion for three distributions. Circles and triangles represent the observed number of ovules and seeds per pod, respectively; Filled circles and circles represent respectively the observed number of seeds and ovules; Solid line and dash line represent respectively the computed number of seeds and ovules. Lines represent the computed number of ovules and seeds per pod for three distributions.

of effective pollen grains (k) was same between 2009 and 2008. The distribution of the number of effective pollen grains did not present significant difference between the two years. The values of parameters for each process are given in Table 4.3. The probability of seed viability (p) was larger in 2009 (Fig. 4.3C). Furthermore, the probability of pod abortion (Bo) was larger in 2008 than 2009 (4.3D), which is due to the whole situation of the number of seeds in the plant. The results indicated that the seed production was better in 2009 than 2008. The variation of the number of ovule per ovary, the number of seeds per pod and pod abortion indicated that the effect of assimilate availability, which could be due to the environmental conditions (rain, temperature and light).

Table 4.3: Estimated parameters of the model for 2008 and 2009.

Description	Distributions	Parameters	Values 2008	2009
Distribution of ovule number	Normal	μ	31	31
Distribution of ovule number	Normal	σ	3.95	2.72
Distribution of pollen number	Log-normal	s	0.875	0.883
Percentage of effective pollen grains	Linear	k	0.798	0.798
Distribution of pollen number	Log-normal	Ao	0.9	0.9
Viability of seeds	Bernoulli	p	0.856	0.88
Pod abortion	Beta	α	3	3
Pod abortion	Beta	Bo	0.25	0.34
	R2		0.95	0.92

4.3.3 Effect of assimilate availability on the estimated parameter values

The data of section 2.7.1 was used to analyze the effect of assimilate availabiligy on the parameter values.

Parameter estimation for clipping ramifications (Variety Mendel)

Observed and computed distributions of the number of ovules (Fig. 4.4A) and seeds per pod (Fig. 4.4B) increased in the clipped plants. As shown in the table 4.4, the mean number of ovules per pod (μ) and the probability of seed viability (p) increased with ramification clipping. In addition, the distribution parameters of the number of pollen grains (s) and the percentage of effective pollen grains (k) were same between the control plants and clipped plants (Fig. 4.4C). The probability of pod abortion differed between the control and clipped plants (Fig. 4.4D). The results indicated that assimilate availability has influence on the number of ovules per ovary, the number of

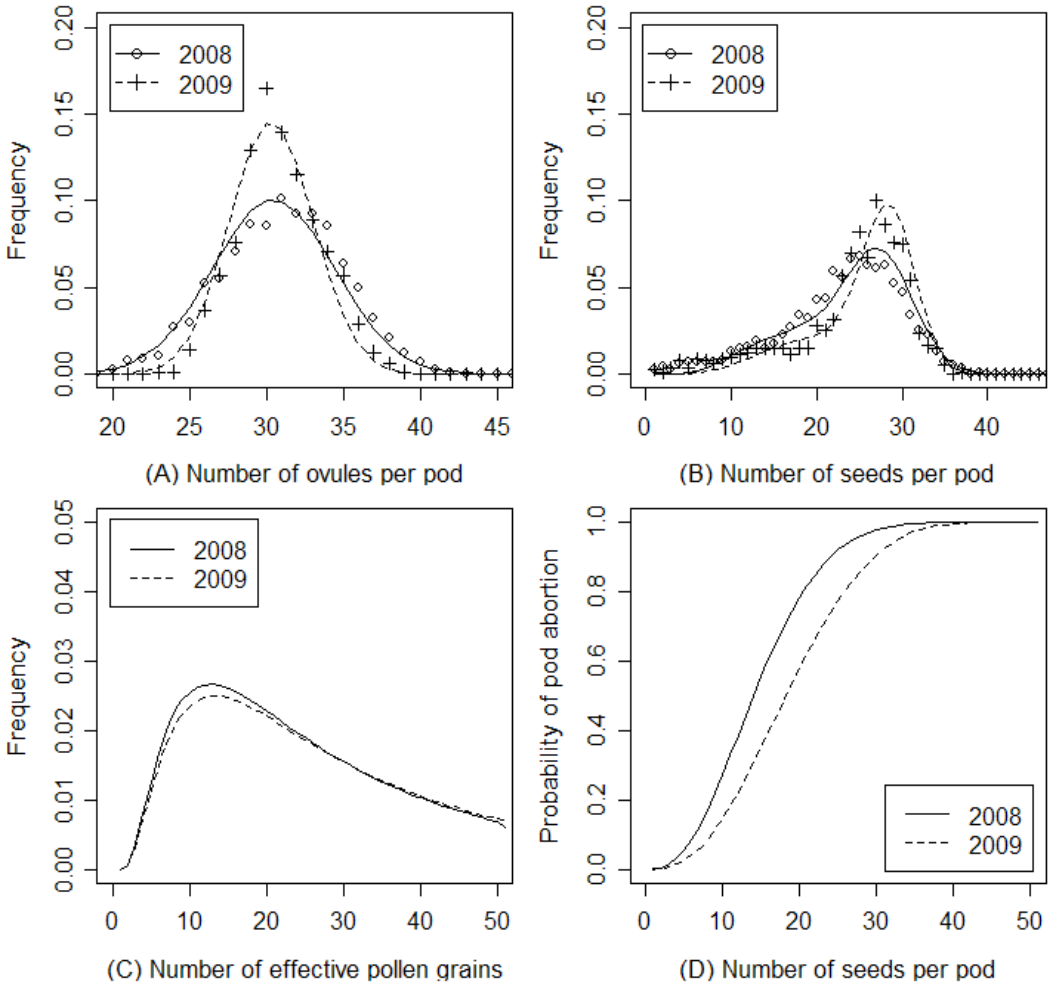


Figure 4.3: Adjusted model and measurements for the number of ovules per flower (A), number of seeds per pod (B), the estimation for the distribution number of pollen grains per flower (C) and the probability of pod abortion according to the number of seeds per pod (D) on the main stem in 2008 and 2009 (Variety: Mendel). Symbols and lines represent observed and computed values, respectively.

seeds per pod and pod abortion, but the distribution of the number of pollen grains per stigma was not impacted by assimilate availability.

Parameter estimation for clipping all the ramification and 20 basal flowers (Variety Pollen)

The distributions of the number of ovules and seeds per pod for the clipped and control plants (Fig. 4.5) did not differ significantly. Likewise, the distribution of the number

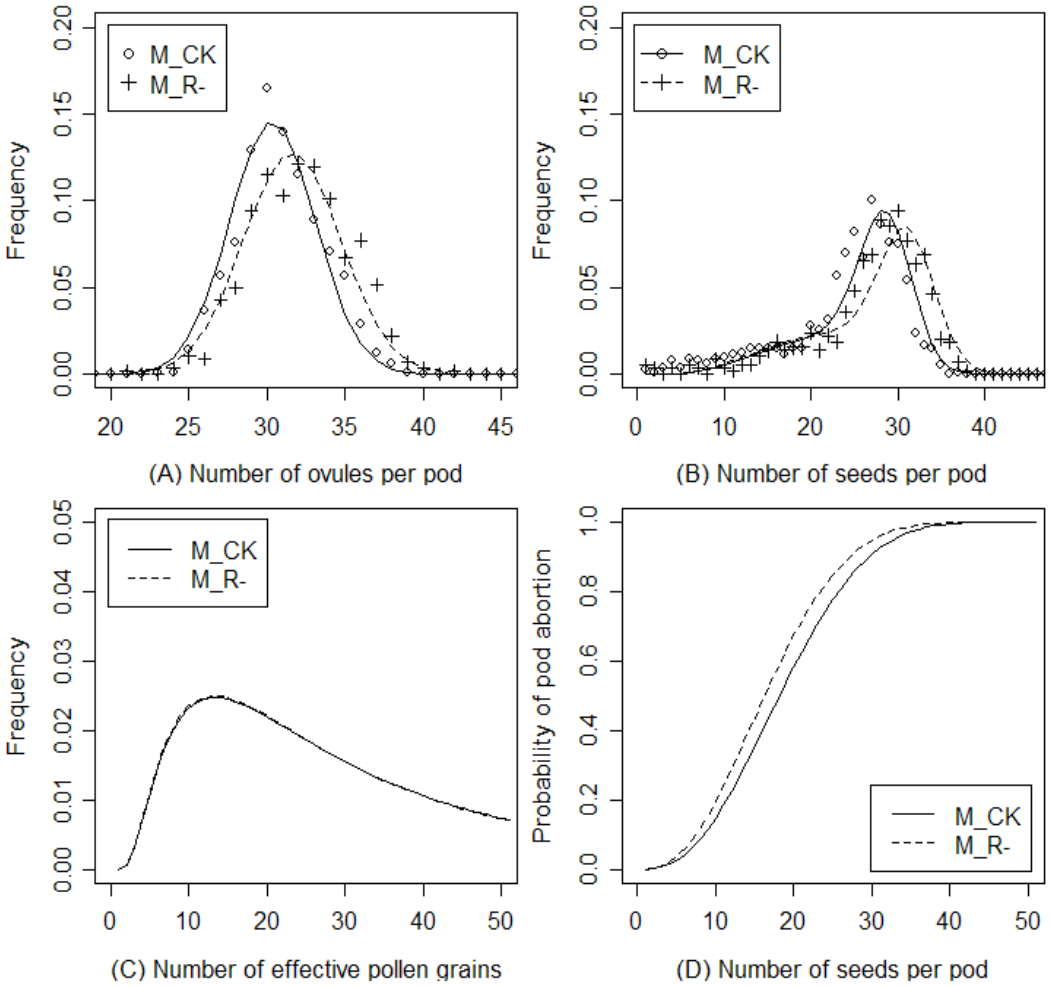


Figure 4.4: Adjusted model and measurements for the number of ovules per flower (A), number of seeds per pod (B), the estimation for the distribution number of pollen grains per flower (C) and the probability of pod abortion according to the number of seeds per pod (D) on the main stem in the control and clipped plants (Variety: Mendel). Symbols and lines represent observed and computed values, respectively.

Table 4.4: Parameter values estimated parameters for each process in the clipping ramifications and control plants.

Description	Distributions	Parameters	Values M_CK	M_R-
Distribution of ovule number	Normal	μ	31	32
Distribution of ovule number	Normal	σ	2.72	3.11
Distribution of pollen number	Log-normal	s	0.883	0.883
Percentage of effective pollen grains	Linear	k	0.798	0.798
Distribution of pollen number	Log-normal	Ao	0.9	0.9
Viability of seeds	Bernoulli	p	0.88	0.93
Pod abortion	Beta	α	3	3
Pod abortion	Beta	Bo	0.34	0.3
	R2		0.92	0.95

of effective pollen grains per flower was not different. However, the probability of pod abortion decreased significantly (Fig. 4.5). The parameter values in the Table. 4.5 gives the same results. The number of ovules and seeds per pod had no difference. This could be due to the decrease of the competition and the supply for assimilates exist simultaneously when clipped all of the ramifications and early flowers. The study of [Yu et al., 2010] showed that the pod can produce assimilates.

Table 4.5: Parameter values estimated parameters for each process in the clipping early flowers and control plants.

Description	Distributions	Parameters	Values P_CK	P_R-'
Distribution of ovule number	Normal	μ	31.4	31.5
Distribution of ovule number	Normal	σ	2.83	2.94
Distribution of pollen number	Log-normal	s	0.894	0.894
Percentage of effective pollen grains	Linear	k	0.9	0.9
Distribution of pollen number	Log-normal	Ao	0.9	0.9
Viability of seeds	Bernoulli	p	0.915	0.923
Pod abortion	Beta	α	3	3
Pod abortion	Beta	Bo	0.23	0.34
	R2		0.99	0.96

4.3.4 Effect of pod rank on estimated parameter values

Data analysis revealed that pod ranks had significant influence on the number of seeds per pod [Wang et al., 2011]. The number of seeds per pod remained constant, and then

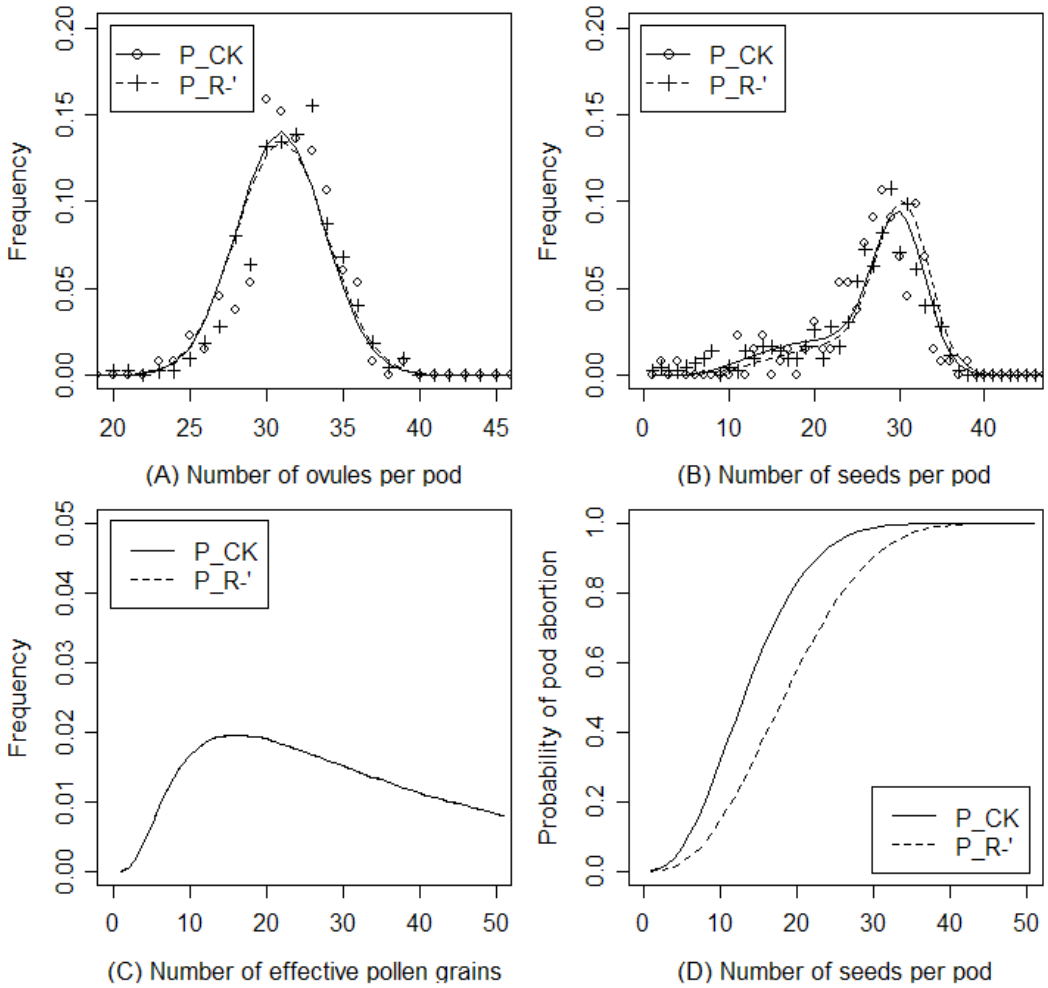


Figure 4.5: Adjusted model and measurements for the number of ovules per flower (A), number of seeds per pod (B), the estimation for the distribution number of pollen grains per flower (C) and the probability of pod abortion according to the number of seeds per pod (D) on the main stem in the control and clipped plants (Variety: Pollen). Symbols and lines represent observed and computed values, respectively.

decreased with higher rank. Thus it is interesting to estimate the variation of parameter values according to the pod rank.

The mean number of ovules per ovary (μ), the probability of seed viability (p) and the parameters of the probability for a pod to survive (Bo) did not differ significantly with the pod rank (Fig. 4.6). The mean value of the parameter μ, p and Bo was 30.9, 0.86 and 0.3, respectively. However, the distribution parameters of the number of pollen grains (s) and the percentage of effective pollen grains (k) varied with the pod rank

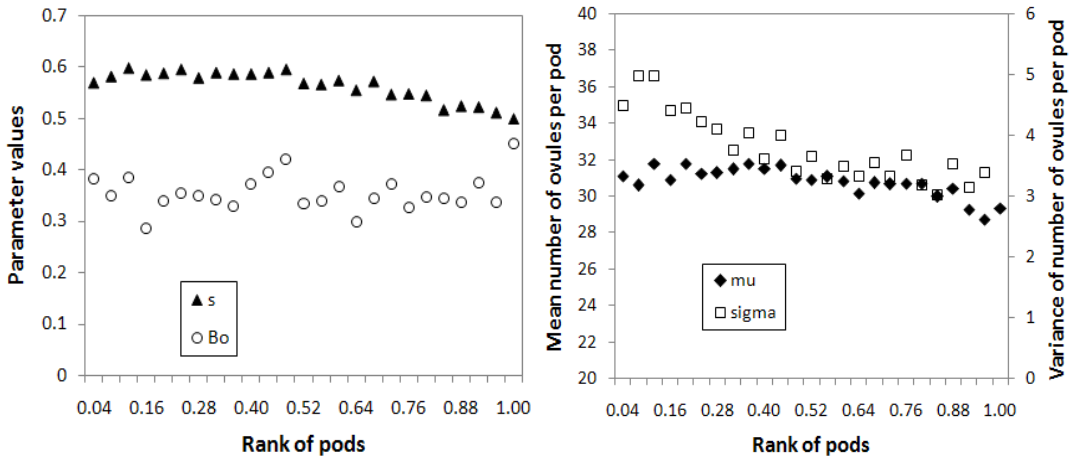


Figure 4.6: Parameter values of model according to the pod rank.

(Fig. 4.6B). The simulated distribution of the number of effective pollen grains can be divided into three stages for the main stem. The effective and total numbers of pollen grains were slightly small for a few ranks, then increased with the pod rank and remained constant, but decreased at the end of the stem.

4.3.5 Effect of inflorescence position on estimated parameter values

To analyse if the distribution of the number of pollen grains differ between inflorescences, the parameter values of each step were set to the same values except the distribution parameter of the total number of pollen grains (s) for the inflorescences R0, R1, R4, R7, R9 and R11. The number of seeds per pod can be well calibarated (Fig. 4.7B).
The mean number of ovules per pod (μ) increased with inflorescences from top to bottom (Table 4.6). The upper the inflorescence is, the larger number of pollen grains has on the inflorescence except ramification R11. The results indicated that inflorescence position had influence on the number of pollen grains, therefore, the number of seeds per pod.

4.3.6 Difference of estimated parameter values for four varieties

The results of parameter estimation for the four varieties under the same conditions are presented in fig. 4.8 and Table 4.7. The mean number of ovules per ovary (μ), the parameters of pollination (s) and (k), seed viability (p) and pod abortion (Bo) varied

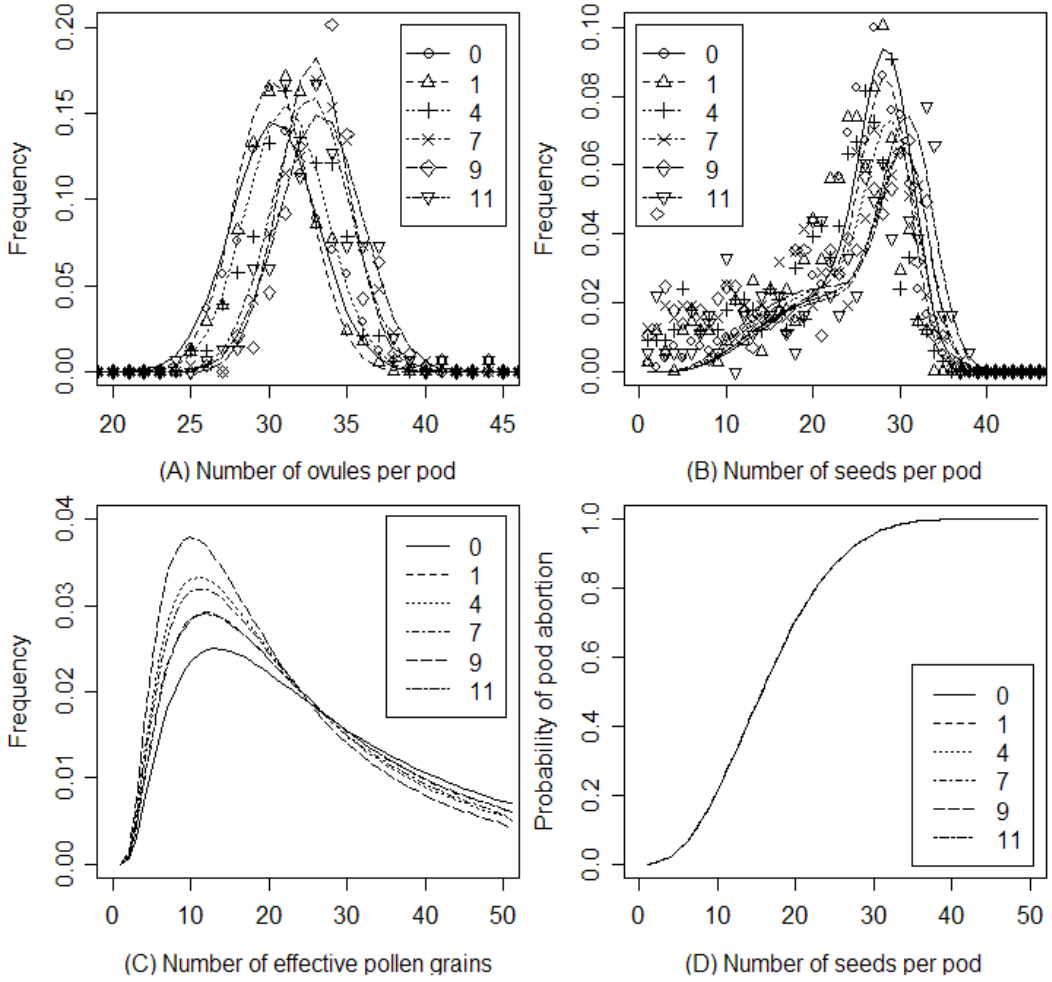


Figure 4.7: Adjusted model and measurements for the number of ovules per flower (A), number of seeds per pod (B), the estimation for the distribution number of pollen grains per flower (C) and the probability of pod abortion according to the number of seeds per pod (D) on the main stem (R0) and ramifications R1, R4, R7, R9 and R11 (Variety: Mendel). Symbols and lines represent observed and computed values, respectively.

Table 4.6: Parameter values of different steps for treatment plants and control ones.

		Values					
Description	Parameters	R0	R1	R4	R7	R9	R11
Distribution of ovule number	μ	30.8	30.7	31.6	33.2	33.4	33.8
Distribution of ovule number	σ	2.72	2.32	2.56	2.48	2.17	2.64
Distribution of pollen number	s	0.883	0.863	0.846	0.852	0.83	0.864
Percentage of effective pollen grains	k	0.798	0.798	0.798	0.798	0.798	0.798
Distribution of pollen number	Ao	0.9	0.9	0.9	0.9	0.9	0.9
Viability of seeds	p	0.88	0.88	0.88	0.88	0.88	0.88
Pod abortion	α	3	3	3	3	3	3
Pod abortion	Bo	0.341	0.341	0.341	0.341	0.341	0.341
	R2	0.92	0.81	0.78	0.73	0.78	0.86

with the variety (Table 4.7).
The Gamin variety had large number of ovules per pod, but smaller number of seeds per pod comparing to the other varieties (Fig. 4.8B).
The distribution of pollination predicted by the model was different between the four varieties (Fig. 4.8C), although they were grown in the same field. The pollination distribution of Gamin variety was significant different from other varieties. The proportion of the number of effective pollen grains was small (0.572) for this variety. In addition, the probability of seed viability (p) was quite small for the Gamin variety according to the estimation results.
Furthermore, the probability of pod abortion differed significantly for the Gamin variety from the other varieties. There was smaller threshold value (14) of pod survival compared to the other varieties. It was 35, 31 and 31 for the variety Exocet, Pollen and Mendel, respectively.

Table 4.7: Estimated parameter values of different processes for each variety.

		Values			
Description	Parameters	Exocet	Gamin	Pollen	Mendel
Distribution of ovule number	μ	37.5	32.9	30.7	29.7
Distribution of ovule number	σ	2.99	2.96	2.66	2.23
Distribution of pollen number	s	1.057	0.938	1.024	1.039
Percentage of effective pollen grains	k	0.997	0.572	0.9	0.809
Distribution of pollen number	Ao	0.9	0.9	0.9	0.9
Viability of seeds	p	0.762	0.476	0.884	0.88
Pod abortion	α	3	3	3	3
Pod abortion	Bo	0.285	0.062	0.269	0.344
	R2	0.9	0.86	0.88	0.9

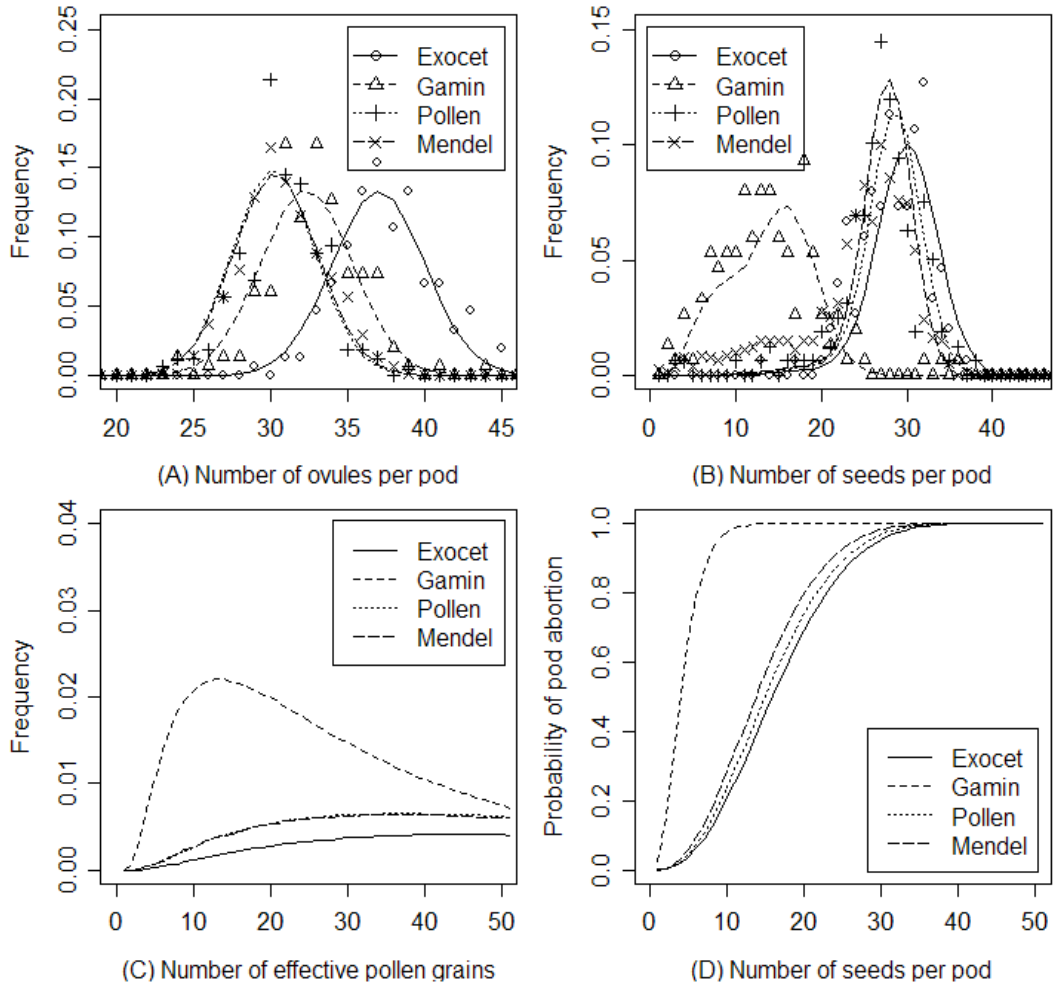


Figure 4.8: Adjusted model and measurements for the number of ovules per flower (A), number of seeds per pod (B), the estimation for the distribution number of pollen grains per flower (C) and the probability of pod abortion according to the number of seeds per pod (D) on the main stem for the four varieties (Exocet, Gamin, Pollen and Mendel). Symbols and lines represent observed and computed values, respectively.

4.4 Conclusion

A model is presented in this paper and it can simulate the abortion of seeds and pods. The parameters of the model were estimated according to the years, clipping treatments, pod ranks, inflorescence positions and four contrasting varieties. The differences of parameter values were analyzed to distinguish the factorst of seed and pod abortion, including ovule fertilization, pollination limitation, competition for assimilates and architectural effects. These factors have different impacts on the abortion of seeds and pods (4.8). According to the estimations, we can draw the following conclusions:

Table 4.8: Variation of parameter values under different conditions for each process.

Parameter	Years	Treatment	Pod ranks	Inflorescences	Variety
μ	Constant	Increase	Constant	Increase	Vary
s	Vary slightly	Constant	Decrease	Decrease	Vary
k	Constant	Increase	Decrease	Constant	Vary
p	Vary	Increase	Constant	Constant	Vary
Bo	Vary	Vary	Vary (no trend)	Constant	Vary

1 The mean number of ovules per flower (μ) was constant between years, but varied with pod positions (within one inflorescence and between inflorescences) and assimilates availability (clipping treatments).

2 The probability of seed viability (p) remained constant with pod ranks and ramifications, but the probability was impacted by assimilate availability (years, clipping treatments). The probability of pod abortion (Bo) was related to the number of seeds it contains.

3 The distribution parameters of the number of pollen grains (s and k) varied with years and pod positions. However, assimilate availability has no effect on this parameter.

Components that determine the number of seeds are the number of ovules per ovary and the percentage of these ovules that develop into seeds. Accordingly, seed abortion could be due to two factors: ovule viability or/and pollination limitation. If the ovule is not viable, even if it can receive a pollen grain, or if the pollen is ineffective, it cannot form a fertilized ovule, thus it leads to seed abortion. Seed abortion results from two periods could due to two factors: ovule viability Bouttier and Morgan [1992b] and pollination limitation [Pechan, 1988].

4.4.1 Ovule viability

[Bouttier, 1990] found that 30-40% ovules lack an embryo sac at flower opening. The success or failure of embryo sac development is already determined at the bud stage of 4 mm [Bouttier and Morgan, 1992b]. According to the study of [Bouttier and Morgan, 1992b], environmental factors, such as temperature, light, nutrient supply, during meiosis II and/or early megaspore differentiation are determining events on seed yields. Within the inflorescence, decreased ovule viability was one of the causes for the lower number of seeds per pod in the apical region compared to the basal region [Bawa and Webb, 1984; Sedgley, 1980]. Lower ovule viability in the distal region of ovaries from the apical region of the inflorescence may result from an insufficient supply of resources leading to intra-ovary competition in later formed buds. The assimilates were intercept by the basal pods [Lloyd, 1980].
The mean number of ovules per ovary increased with inflorescence from top to bottom along the main stem, and the mean increased with clipping treatment. The results could be due to assimilate availability [Bouttier, 1990]. The supply and allocation of assimilates between the organs within the plant vary with the time. This could lead to the difference of assimilates obtained by pods appear in different time and position [Keiller and Morgan, 1988; Stephenson, 1981]. Furthermore, clipping treatment can reduce the competition for assimilates, which results in the increase of the number of ovules per ovary.
Furthermore, the number of ovules per ovary varies with the variety, as indicated by the results of the four varieties. This phenomenon is well known in other plants [De Reffye, 1974; de Reffye et al., 1978; Falque et al., 1995]. Normally, the number of ovules is much larger than the final number of seeds, therefore, it is not a limiting component [Ancha, 1988; Mendham et al., 1981b; Pechan and Morgan, 1985]. The numbers of seeds that develop in pods are mainly limited by the failure of fertilization [Pechan, 1988].

4.4.2 Pollination limitation

Poor pollination is often cited [Teixeira et al., 2006] to explain low seed set, and the stochastic pollinator environment is often stressed as an important cause of pollen limitation Price et al. [2005]. The quantity of pollen in the air is strongly influenced by weather conditions, such as wind, temperature and precipitation [Gruber and Claupein, 2007], as wind pollination is important for the fertility of oilseed rape. De Reffye [de Reffye et al., 1978] noted that pollination is a random process which varies with the season and the genotype in cacao tree. The decrease in the number of seeds per pod observed at the top of the main stem could be the consequence of the decreasing number of flowers or/and inefficient pollinators. Indeed, most flowers develop into pods at the end of the flowering period and the main stem gradually stop growing, which lead to a lower number of flowers in the field, and hence a reduced amount of pollen grains for late flowers. Furthermore, the amount of pollen fluctuated greatly from day to day and

varied with the stage of flowering of the crop [Williams, 1984]. The model estimated the distribution of the number of effective pollen grains per flower in different pod ranks and inflorescence positions. The results were consistent with the conclusions above. As the main stem expands first, followed by the ramifications from top to bottom, the position that flower located determines the time of its flowering and pod setting. During flowering period, few pollen grains were caught during early and late flowering and most during peak flowering [McCartney and Lacey, 1991]. The estimation for four varieties indicated that pollination distribution varied largely, especially for the Gamin variety. One output of the model is the pollination law that is not easy to measure experimentally. The distribution of the number of pollen grains deposited was in coherent with the result of Mesquida and Renard [Mesquida and Renard, 1982; 1983; Mesquida et al., 1988]. He studied the distribution of pollen grains in oilseed rape and found most flowers can receive enough pollen grains to fertilize the ovules. The model allows us to deduce the law of pollination deposited and it is a kind of validation.
[Pechan, 1988] and [Ancha, 1988] found that pollen germination was not the factor leading to seed aboriton Araujo et al. [2007]. The failure of ovule viability and the pollen tube penetrating the ovule are the important factors of seed abortion. The decreases of the number of seeds per pod and of the number of effective pollen grains at the end of the inflorescence could be due to the reduced ability of ovule fertility, or the decrease of the quantity and quality of pollen grains.
In addition, many ovules do not resume growth after pollination and degenerate by 2 days after flower opening [Bouttier and Morgan, 1992b]. That could be due to the triple fusion does not take place (no endosperm), which leads to seeds were 'starved to death' from the lack of nutrients [Pechan, 1988]. Some seeds stop growing after a few days of development. It could be a result of delayed development and consequently reduced ability to compete with the more developed seeds for available nutrients. However, the second series of abortions does not affect many seeds [Pechan and Morgan, 1985]. The above evidence suggests that the maturity and/or the receptivity of the ovules at the time of flower opening may be important in determining seed set [Ancha, 1988]. Thus, the opened flower stage is critical for seed set.

4.4.3 Competition for assimilates

Brassica napus is a species with a complex reproductive architecture, yield results from the product of the number of ramifications, number of pods per ramification, number of seeds per pod [Mendham et al., 1981a]. The plant is very plastic, and there may be compensatory mechanisms between different components that are linked to source-sink relationships [Wang et al., 2011]. The competition for assimilates within one plant leads to a trade-off between inflorescences. The rhythm of pod setting for ramifications was found to be slower than for the main stem. This could be due to pod setting on ramifications is subject to higher competition for assimilates than on the main stem

because of the internode expansion and pod setting on all ramifications within a short period [Jullien et al., 2010]. The probability of seed viability increased in the clipping ramifications or basal flowers plants indicated that the competition for assimilates has effect on seed and pod production. [Tayo and Morgan, 1979] studied the impact of shading or leaf removal on the number of pods on the main stem. They concluded that irrespective of the developmental stages over which shading took place, reductions in the number of pods occurred on the terminal inflorescences. The number of pods per plant was significantly reduced when the shading was applied at anthesis, although the reduction in the number of pods was partially compensated by an increase in the number of seeds per pod in the basal pods. Leaf removal treatments led to more severe reductions in the number of pods, pod yield and seed yield than the shading treatments. Furthermore, [Pechan and Morgan, 1985] found that defoliation on the terminal inflorescence at anthesis causes a significant reduction in the weight of pods per plant as a result of reducing the weight of the individual pods. These studies suggest that the competition for assimilates within one inflorescences may be due to the intercept of assimilates of the basal pods.
Competition for assimilates should be one cause leading to the abortion of pods. When the upper pods start growing, they are simultaneously confronted to a higher competition for assimilates and a decrease in the sources [Stephenson, 1980; Udovic and Aker, 1981]. And there is competition among the ovaries of an inflorescence, as speculated by the resource competition hypothesis [Arathi et al., 1999; Lee, 1988; Stephenson, 1980]. Model parameter estimation indicated that the resource competition is not visible at the level of the seed (p constant according to the pod rank), but rather at the pod level. The probability of pod abortion is correlated to the number of seeds it contains. The amount of assimilates is regulated at the level of single flower and fruit, particularly for the determination of flowers, the development of ovaries and the maturation of fruit. At each stage, the initiation or expansion of an organ requires an amount of available resources above a certain threshold [Ganeshaiah et al., 1986]. If a pod with too few seeds, it will abort. This phenomenon could be related to a lack of available assimilates. Hence, assimilate is continually adjusted to the resources available at each developmental stage.

4.4.4 Architectural effects

The position of pods within one plant varies due to the complex structure in WOSR. The difference of pod positions results in the difference of initial time of pods [Egli and Bruening, 2006]. The time of flowering and pod setting differ between the pods within one inflorescence and between inflorescences within one plant. The basal pods and the apical inflorescences flower and develop first. The time is closely related to the supply of assimilates [Gabrielle et al., 1998] and pollination conditions [Kang and Primack, 1991]. This could be lead to the variation of the number of ovules and seeds per pod in WOSR.

In conclusion, the model allows us to reproduce the steps of flower fertility on WOSR with a few parameters and probabilistic distributions. Model can simulate the phenomena of observations and allow us to deduce the distribution of the number of pollen grains in a flower and to identify the factors that influence the yield, which include ovule viability, resource competition and pollination limitation and architectural effects. Model parameters are useful as selection criteria, to study variability of seed production, or to estimate the contribution of genetic and environmental factors to seed abortion, given a relevant experimental design.

4.5 Stability of model parameters - Jackknife and Bootstrap resampling

In statistics, resampling is used to test the stability of model parameters by using random subset, such as jackknifing, bootstrapping and cross validation.
Jackknifing was introduced by [Quenouille, 1949], which is used in statistical inference to estimate the bias and standard error (variance) of a statistic, when a random sample of observations is used to calculate it. The basic idea behind the jackknife variance estimator lies in systematically recomputing the statistic estimate leaving out one or more observations at a time from the sample set. From this new set of replicates of the statistic, an estimate for the bias and an estimate for the variance of the statistic can be calculated [Li et al., 2008].
Bootstrapping is a statistical method for estimating the sampling distribution of an estimator by sampling with replacement from the original sample [Chaudhury et al., 1998], most often with the purpose of deriving robust estimates of standard errors and confidence intervals of a population parameter like a mean, median, proportion, correlation coefficient or regression coefficient [Sahinler and Topuz, 2007]. It may also be used for constructing hypothesis tests. It is often used as a robust alternative to inference based on parametric assumptions when those assumptions are in doubt, or where parametric inference is impossible or requires very complicated formulas for the calculation of standard errors.
The bootstrap and the jackknife estimate the variability of a statistic from the variability of that statistic between subsamples, rather than from parametric assumptions. The jackknife is a less general technique than the bootstrap, and explores the sample variation differently. However, the jackknife is easier to apply to complex sampling schemes, such as multi-stage sampling with varying sampling weights, than the bootstrap. The jackknife and bootstrap may in many situations yield similar results. But when used to estimate the standard error of a statistic, bootstrap gives slightly different results when repeated on the same data, whereas the jackknife gives exactly the same result each time (assuming the subsets to be removed are the same).
Whether to use bootstrap or jackknife may depend more on non-statistical concerns

but on operational aspects of a survey [Muller, 2005]. The bootstrap provides a powerful and easy way to estimate not just the variance of a point estimator but its whole distribution, thus becoming highly computer intensive. On the other hand, the jackknife (originally used for bias reduction) only provides estimates of the variance of the point estimator. This can be enough for basic statistical inference (e.g. hypothesis testing, confidence intervals). Hence, the jackknife is a specialized method for estimating variances whereas the bootstrap can be applied to both variance and distribution estimation problems. However, the bootstrap variance estimator is not as good as the jackknife in terms of the empirical results. Furthermore, the bootstrap variance estimator usually requires more computations than the jackknife [Spiegelman and Park, 2007]. Thus, the bootstrap is mainly recommended for distribution estimation. In this section, we used the jackknifing and bootstrapping method to test the uncertainty we have on our parameter values.

4.5.1 Jackknife resampling

Introduction

The jackknife resampling method is used to perform an investigation of the stability or uncertainty of the fitted model [Li et al., 2008; Sahinler and Topuz, 2007]. By resampling with omission of the original sample, the stability of the parameters derived from the observations can be determined.
Due to the large size of our data sample, the deleted-1 is too computationally intensive, thus the delete-q jackknife approach [Efron and Tibshirani, 1993] is used in our study. Assume a data sample of size n is divided into g groups of size $q(n = gq)$, denoted by $X = x_1, x_2, \ldots, x_{g-1}, x_g$. The aim is to estimate the parameter $\hat{\theta} = f(x)$. The delete-q jackknife estimate of standard error of $\hat{\theta}$ is obtained by deleting selected groups of observations at a time, and computing $\hat{\theta}_i$ using the remaining sampled data, the equation is given by 4.19:

$$\hat{\theta} = f(x_1, x_2, \ldots, x_{i-1}, x_{i+1}, \ldots, x_{g-1}, x_g) \tag{4.19}$$

This process is repeated for $i = 1, 2, \ldots, g-1, g$ until each group has been deleted once and only once, there by generating g jackknife estimates of $\hat{\theta}$. The mean and standard error of the jackknife estimate are given by 4.20 and 4.21:

$$\bar{\hat{\theta}} = \frac{1}{g}\sum_{i=1}^{g}\hat{\theta}_i \tag{4.20}$$

$$STD = [\frac{1}{g-1}\sum_{i=1}^{g}(\hat{\theta}_i - \bar{\hat{\theta}})^2]^{\frac{1}{2}} \tag{4.21}$$

The 95%($\alpha = 0.05$) confidence intervals of mean with the unknown population variance are estimated by using the following equation 4.22:

$$\bar{\hat{\theta}} - t_{g-1,\frac{\alpha}{2}} \frac{g-1}{g} STD < \theta < \bar{\hat{\theta}} + t_{g-1,\frac{\alpha}{2}} \frac{g-1}{g} STD \tag{4.22}$$

where $t_{g-1,\frac{\alpha}{2}}$ is the critical value of t-distribution with probability $\frac{\alpha}{2}$ the right-tailed for $g-1$ degrees of freedom [Sahinler and Topuz, 2007]. The factor $\frac{g-1}{g}$ have been chosen such that STD is unbiased variance estimator of the standard deviation [Duchesne and MacGregor, 2001].

Results

The data of 2008 was used with 1800 pods, corresponding to 45 plants with 40 pods per plant. The parameters were estimated, with removing one plant (40 pods) from the whole sample at each step for the jackknife method. Table 4.9 gives the mean of parameter values, their standard deviation and their coefficient of variation (CV), where $n = 1800, g = 45, q = 40, t_{44,\frac{\alpha}{2}} = 2.015$. The CVs indicate a quite good stability for all the parameters, the less accurate ones being the parameters σ related to the variance of the number of ovules per pod and the parameters (Bo) related to the probability for a pod to survive according to its number of seeds.

Table 4.9: The summary statistics of the parameter values for jackknife subsample ($n = 1800, g = 45, q = 40, t_{44,\frac{\alpha}{2}} = 2.015$)

Parameter	mean	STD	CI_min	CI_max	CV (%)
μ	31.3	0.147	31.0	31.6	0.47
σ	4.1	0.135	3.8	4.3	3.32
s	0.924	0.004	0.916	0.931	0.423
p	0.846	0.006	0.834	0.857	0.708
Bo	0.211	0.005	0.201	0.221	2.3

4.5.2 Bootstrap resampling

Introduction

A dataset of size n has possible $2^n - 1$ non-empty subsets; however, the jackknife resampling uses only n of them. Thus, it can be seen that there is further scope for resampling and the jackknife resampling may be improved upon by obtaining estimates from more than n subsets. For this purpose, the bootstrap resampling was introduced by Efron [Efron, 1979]. The idea behind the bootstrap resampling is to randomly sample the

dataset a very large number of times (b times), in a similar manner to Monte Carlo simulation.
The assumption is that each data point is a valid member of the total sample and that at any time, there is an equal probability of 'picking' any of the data values in the original sample. Thus, new and equally valid resampled datasets can be created by picking from the original sample at random.
Bootstrap resampling is achieved by randomly selecting n data points, with replacement, from the original observed random sample. Therefore, it is possible that in any sample of n data points, some of the original data points can appear twice or more and some of the original data points may not appear at all. All bootstrap replicates (samples) have the same length as the original samples and each of the b bootstrap replicates can provide an estimator $\hat{\theta}$. The spread in the estimators formed from these resampled datasets then provide information on the stability of the estimator with respect to different possible outcomes represented by the bootstrap replicates. However, resampling with replacement may lead to unrealistic bootstrap samples. Therefore, a large number of replicates are generally recommended.
The mean and standard error of the bootstrap estimate are given by 4.23 and 4.24:

$$\bar{\hat{\theta}} = \frac{1}{b}\sum_{i=1}^{b}\hat{\theta}_i \tag{4.23}$$

$$STD = [\frac{1}{b-1}\sum_{i=1}^{b}(\hat{\theta}_i - \bar{\hat{\theta}})^2]^{\frac{1}{2}} \tag{4.24}$$

The 95%($\alpha = 0.05$) confidence intervals of mean with the unknown population variance are estimated by using the following equation 4.25:

$$\bar{\hat{\theta}} - z_\alpha STD < \theta < \bar{\hat{\theta}} + z_\alpha STD \tag{4.25}$$

where z_α is the critical value of z-distribution with probability α the right-tailed for $b-1$ degrees of freedom [Sahinler and Topuz, 2007].

Results

Table 4.10 gives the mean of parameter values, their standard deviation and their coefficient of variation (CV), where $n = 1800, b = 100, z_\alpha = 1.645$. The parameter σ related to the variance of the number of ovules per pod and the parameters (Bo) related to the probability for a pod to survive according to its number of seeds had large CVs.

4.5.3 Conclusion

The results indicated that the CVs were larger with bootstrap resampling than jackknife resampling, which results from the resample method. Jackknife method deletes $q(q =$

Table 4.10: The summary statistics of the parameter values for bootstrap subsample ($n = 1800, b = 100, z_\alpha = 1.645$)

Parameter	mean	STD	CI_min	CI_max	CV (%)
μ	31.3	0.74	30.0	32.5	2.36
σ	4.0	0.45	3.27	4.76	11.2
s	0.923	0.01	0.906	0.939	1.07
p	0.848	0.02	0.815	0.88	2.36
Bo	0.21	0.009	0.195	0.225	4.4

40) samples from the whole sample for each time, the subsample is more similar with the whole population. While bootstrap method randomly select the samples from the whole sample, some samples could not be selected, but some samples could be selected several times or more, this could be lead to the large difference from the population. However, the results obtained by jackknife resampling and bootstrap resampling are consistent. The CV of the variance of the number of ovules per pod (σ) was larger compared to the other parameters. Furthermore, the parameter Bo had big variation, which could result from the number of seeds per pod. This could be due to the different subsamples, which have different number of ovules and seeds per pod. Thus, we can conclude that sample set has an effect on the estimations of the variance of the number of ovules and the probability for a pod to survive, but sample set has no effect on the estimation of the distribution of pollen grain number and the probability of seed viability.

4.6 Model comparison

4.6.1 A pollination and fertilisation model for multi-seeded fruit and its application to kiwifruit

Lescourret [Lescourret et al., 1999] developed a model describing flower pollination and ovule fertilisation. The outcome of the model varies with the climate, the number and phenology of flowers in an orchard, the planting scheme and the choice of pollenizers. The model takes into account the presence in the orchard of various pollenizer groups. The model was applied to kiwifruit, which is a dioecious species.
The fertilization of a kiwifruit flower can be viewed as the combination of four processes:

1 Deposition of pollen grains on the stigmas during the effective pollination period of the flower

2 Selection of fertile pollen grains, i.e. the grains that produce tubes able to bring the male gametes to the ovules

3 Fertilization of ovules conditional on the presence of N ovules in the ovary

4 Selection of fertile ovules.

- The first process a Poisson-distributed deposition of pollen on the stigmas of flowers during the effective pollination period of these flowers. The Poisson distribution is currently employed to count events or things randomly dispersed in time or space, as it can be assumed here for pollen grains in the space occupied by flowers. For the sake of simplicity, they suppose that the population of pollenizers is homogeneous in regard to pollen fertility and pollen production per flower. The intensity λ of this Poisson process is supposed to depend on the date of anthesis of the flower to be pollinated with respect to the temporal pattern of pollen availability in the orchard. They assumed that pollen release is uniform balanced during the effective pollination period. Pollen production depends on the number and time-distribution of flowers that open, and on the number of pollen grains produced per flower p, which is considered as a fixed input value and can vary with the cultivar. Pollen reception is a function that should describe the part of the pollen produced by a source that is transported on the target, according to various features among which the distance between the source and the target. This function may be different according to the species. They suggest a function for the case of kiwifruit.

- The model assumed that the second process is binomial distribution with parameter f (pollen fertility), considering that at each trial (selection of a pollen grain), either of two exclusive events can take place, i.e. the pollen grain is fertile (with a probability f) or not fertile (with a probability $1 - f$).

- For the third process, a basic hypothesis, formulated by Falque et al. [Falque et al., 1995], is that pollen tubes reach ovules in a similar way whether or not these ovules have already been reached by another pollen tube. According to Falque et al. [Falque et al., 1995], it results that the probability that a fertile pollen tube does not reach a given ovule i among a total of N ovules present in the ovary is $1 - \frac{1}{N}$. Then, combining the Poisson process of intensity λ and the binomial process of parameter f (pollen fertility) leads to the formulation of the third process, i.e. the calculation of the probability that ovule i is fertilized given a total of N ovules in the ovary [Lescourret et al., 1998] 4.26:

$$P(i \quad fertilized|N) = 1 - e^{-\frac{\lambda f}{N}} \tag{4.26}$$

- The fourth process is binomially distributed with parameter F (ovule fertility), Ovule i fertilization by pollen and ovule fertility being assumed independent, the probability that i is fertile and fertilized is $F(1 - e^{-\frac{\lambda f}{N}})$.

The result of the combination of the four processes is viewed as binomially distributed, and the probability that n ovules out of N develop into mature seeds is thus 4.27:

$$P(n|N) = C_N^n[F(1-e^{-\frac{\lambda f}{N}}]^n[F(1-e^{-\frac{\lambda f}{N}}]^{N-n} \tag{4.27}$$

provided that ovules in a flower are independent of each other with regard to fertilization.
In our model, we chose the smaller values of the number of ovules and pollen grains as the number of fertilized ovules. That is to say, the ratio of ovule and effective pollen grain was 1:1. But for most species, one pollen may not enough for fertilizing one ovule [Falque et al., 1995]. If we let each pollen to randomly choose which ovule to fertilize, we can estimate the parameters to fit the distribution curves of the number of ovules and seeds per pod by setting a maximum value of the number of pollen grains. According to this method, we can compute the distribution of the number of fertilized ovules. Thus, we can improve our model to compute the number of seeds per pod.
Calculation of the probability that ovule i is fertilized given a total of Y ovules in the ovary and a total amount of X pollen grains on the stigmas: X, Y, Z denotes that the numbers of pollen grains, ovules and fertilized ovules, respectively. The equation to compute the probability of fertilized ovules is 4.28:

$$P(Z=z) = \sum_{j=1}^{X} C_Y^i \frac{j}{Y}(S_{j-1}^{X-1} + S_j^{X-1}) \tag{4.28}$$

4.6.2 Estimation results

We tried to change the maximum number of pollen grains to obtain the best estimation results. We found that when the maximum number of pollen grains was set to 100, the distributions of the number of ovules and seeds per pod can be well calibrated, as shown in the Fig. 4.9. The probability with small number of pollen grains was quite small, the maximum probability of the number of pollen grains was 0.03 for this model and 0.06 for our model. The result is consistent with that obtained with our model, in which the distribution of the number of efficient pollen grains was given. Besides, the distribution of in 2009 was better than 2008, which is in accordance with the result of our model.

4.6.3 Conclusions

The estimation for the distribution of pollen grain number was improved by comparing our model to the model of flower fertility in kiwifruit developed by Lescousrret et al. The flower fertility of model described the distribution of pollen grain number as a Poisson distribution, and computed the fertility of pollen using Binomial distribution, then computed the number of fertilized ovules. The model developed in the thesis

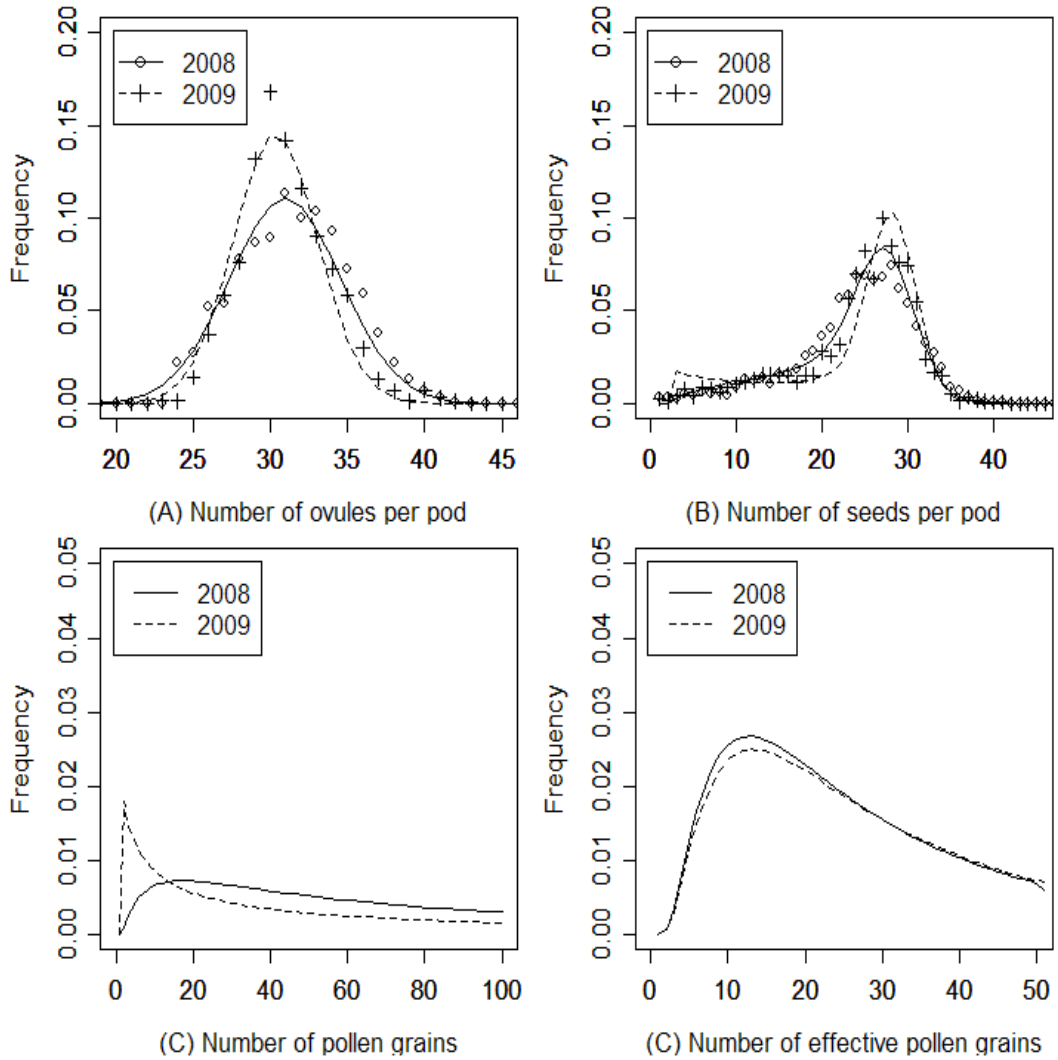


Figure 4.9: Adjusted model and measurements for the number of ovules per flower (A), number of seeds per pod (B), the estimation for the distribution number of pollen grains per flower using the flower fertility of model (C) and the estimation for the distribution number of effective pollen grains per flower using our model (D) on the main stem in 2008 and 2009 (Variety: Mendel). Symbols and lines represent observed and computed values, respectively.

assumed that the ratio of ovule and pollen was 1:1. The number of fertilized ovules was the smaller value of them. However, the studies in other species found that one pollen grain might not be enough to fertilize one ovule. Thus, we introduced one parameter k to estimate the proportion of effective pollen grains in the model, in turns, to compute

the distribution of pollen grain number.
According to the estimations, it is better if we can let one pollen to randomly select one ovule to fertilze. However, the computation is time-consuming. Comparing to the results using the model developed in the thesis, the results are not very large improvement. Therefore, we choose to introduce a parameter k to compute the effective pollen grains in our model, as mentioned before. The assumation that one pollen is sufficient to fertile one ovule is approriate to estimate the distribution of the number of pollen grains. Our model can well calibrate the distribution of the number of ovules and seeds per pod.

4.7 Application to other species

The angiosperms, or flowering plants, are one of the major groups of extant seed plants and arguably the most diverse major extant plant group on the planet, with at least 260,000 living species classified in 453 families [II, 2003; Judd et al., 2002]. They can be small herbs, parasitic plants, shrubs, vines, lianas, or giant trees. There is a huge amount of diversity in reproductive morphology. Despite their diversity, angiosperms are clearly united by a suite of synapomorphies including:

1 An ovary consists of ovules that are enclosed within a carpel, and the stigma, a structure where pollen lands and germinates on it;

2 Stamens with two pairs of pollen sacs;

3 Double fertilization, which leads to the formation of an endosperm (a nutritive tissue within the seed that feeds the developing plant embryo);

4 Features of gametophyte structure and development [Doyle and Donoghue, 1986; Soltis and Soltis, 2004; Soltis et al., 2004].

These characteristics include flowers, endosperm within the seeds, and the production of fruits that contain the seeds. Thus, we try to use our model of flower fertility to the other species, such as soybean and cacao tree.

4.7.1 Soybean

Plant

The soybean (*Glycine max* L. Merr., family Leguminosae), the height of the plant varies from below 20 cm up to 2 metres. The pods, stems, and leaves are covered with fine brown or gray hairs. The leaves are trifoliolate, having 3 to 4 leaflets per leaf, and the leaflets are 6-15 cm long and 2-7 cm broad. The leaves fall before the seeds are mature. The inconspicuous, self-fertile flowers are borne in the axil of the leaf and are white,

pink or purple.
The seeds are borne, 1-5 (usually 2-4) to a pod, the 3-15 pods are in a cluster on the short seed stalk in the rachis or base of the leaf. A productive plant may have as many as 100 seed clusters. The seeds are mechanically harvested after the plant sheds its leaves as it matures.

Inflorescence

There may be from 1-35 purple or white florets, 3-8 of an inch long, on each short raceme or flower cluster. A single plant may bear as many as 800 florets, but may set only 13-57 percent. The floret has the characteristics and shape of many other legume flowers - a large standard petal, two small wing petals, and a keel petal that encloses the staminal column (Fig. 4.10). The calyx is relatively large in proportion to the flower or even to the calyx of other legumes. Each floret is capable of producing a bean pod. Southern grown cultivars stop growing when flowering begins. Flowering usually continues for 4 to 6 weeks. There may be one-half million florets per acre. There are no extrafloral nectaries (Jaycox 1970).

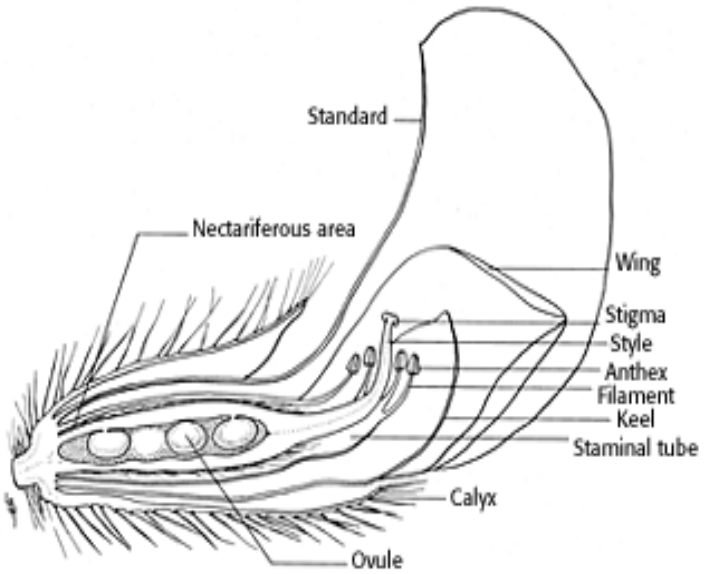


Figure 4.10: Structure of soybean flower (From website)

The soybean is considered to be self-fertile and not benefited by insect pollination [Rubis, 1970]. Soybean flowers attract relatively few bees. Pollination and fertilization is usually accomplished before the flower opens. Therefore, we do not need to consider the distribution of the number of pollen grains per flower. Thus, the computation of

the number of seeds per pod can be described as three steps: (1) the number of ovules per pod; (2) the viability of seeds; (3) the abortion of pods.

Estimation results

The number of ovules and seeds per pod can be computed very well in soybean using the model (Fig. 4.11).

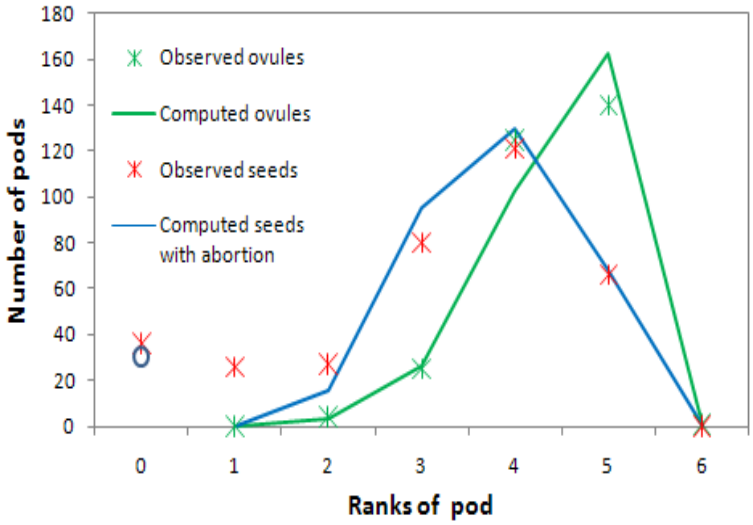


Figure 4.11: Estimations and measurements for the number of ovules and seeds per pod in soybean

4.7.2 Cacao

Cacao

Cacao (*Theobroma cacao* L., Sterculiaceae) is a tropical tree with its center of diversity located in the Amazon basin [Lanaud, 1986], and it is now cultivated in the humid tropical regions of West Africa, Latin America and Aisa. Cacao flowering is usually abundant, particularly in full sun, with up to 125000 flowers per tree each year [Lachenaud and Mossu, 1985]. Cacao inflorescences are grouped in flower cushions located on the tree trunk and branches. The hermaphrodite flowers last only 1 day, as for many tropical species [Bawa, 1983]. Each flower contains five styles connate at their base, which end in a five-branched stigma, and the ovary contains 40-65 ovules [Lachenaud, 1991]. Pollination intensity (PI), the number of pollen grains received per stigma, was monitored in Ivory Coast [de Reffye et al., 1978; Parvais et al., 1977]. These studies indicate that 53-76% of the stigmas were void of pollen. [Falque et al., 1995] also examined the

distribution of the number of seeds per fruit and concluded that flower abortion and low number of seeds per pod were consequence of the small number of fertilized ovules resulting from insufficient pollination.
In this thesis, we consider the fertility of cacao flowers as follows: (1) number of ovules per flower; (2) number of pollen grains per style; (3) number of fertilized ovules; (4) seed viability; (5) Pod abortion.

Source of data set

The data come from the paper of Pauline [Paulin, 1981]. The study was conducted in the station the IFCC Bingerville. The cultivar is UPA 620. They measured the number of ovules per pod, the number of pollen grains per style and the number of seeds per pod. Thus, we can estimate the parameter values using the data. Four groups of data in cacao tree were used to estimate the parameter values using the model (Fig. 4.12). The distribution curves of the number of seeds per pod (Fig. 4.12 A, B, C) illustrate the impact of the pollination on seed filling and pod yield in natural pollination. Fig. 4.12 D shows the distribution curve of the number of seeds per pod with hand pollination for the UPA clone 620.
The curve shown in Fig. 4.12 A is positively skewed unimodal, the plot had been subject to a very inadequate pollination (Pareto: a=2.10). a is the index of scarcity of pollen for the Pareto distribution. The larger a is, the less the number of pollen grains per style. No flower has received enough pollen to ensure that all ovules from the ovary are fertilized. The curve in Fig. 4.12 B is bimodale, the value of a was reduced (a=0.93), the pollination conditions are better, but still insufficient, it has a center peak saturation of 46, which shows a certain proportion flowers were pollinated properly: the pollen is deposited in quantities exceeding the average number of ovules. Figure 4.12 C shows that natural pollination was good: the curve is a unimodal negative asymmetry, the great majority of flowers had been pollinated enough, the scarcity index is low (a=0.35), little flowers with insufficiently pollinated. In the Fig. 4.12 D, there was sufficient pollen grains on all the styles and the scarcity index tends to 0 (a=0.08), we observed a peak very marked. All pods are properly filled seed, the optimal harvest is here. The peak was displaced very significantly, from 47 to 51. The fertility has been completed well with P=0.95. With hand pollination, they optimized the fertility of pollen (viability, compatibility) by controlled choice pollen. Thus, under the same number of pollinated flowers, the quality of pollination (the number of flowers receiving large aggregates) is an essential component.

Estimation results

We can see that our model can compute the number of seeds per pod well when seed production is better (4.12 C,D). However, if seed production is not good, the model can not compute the number of seeds well.

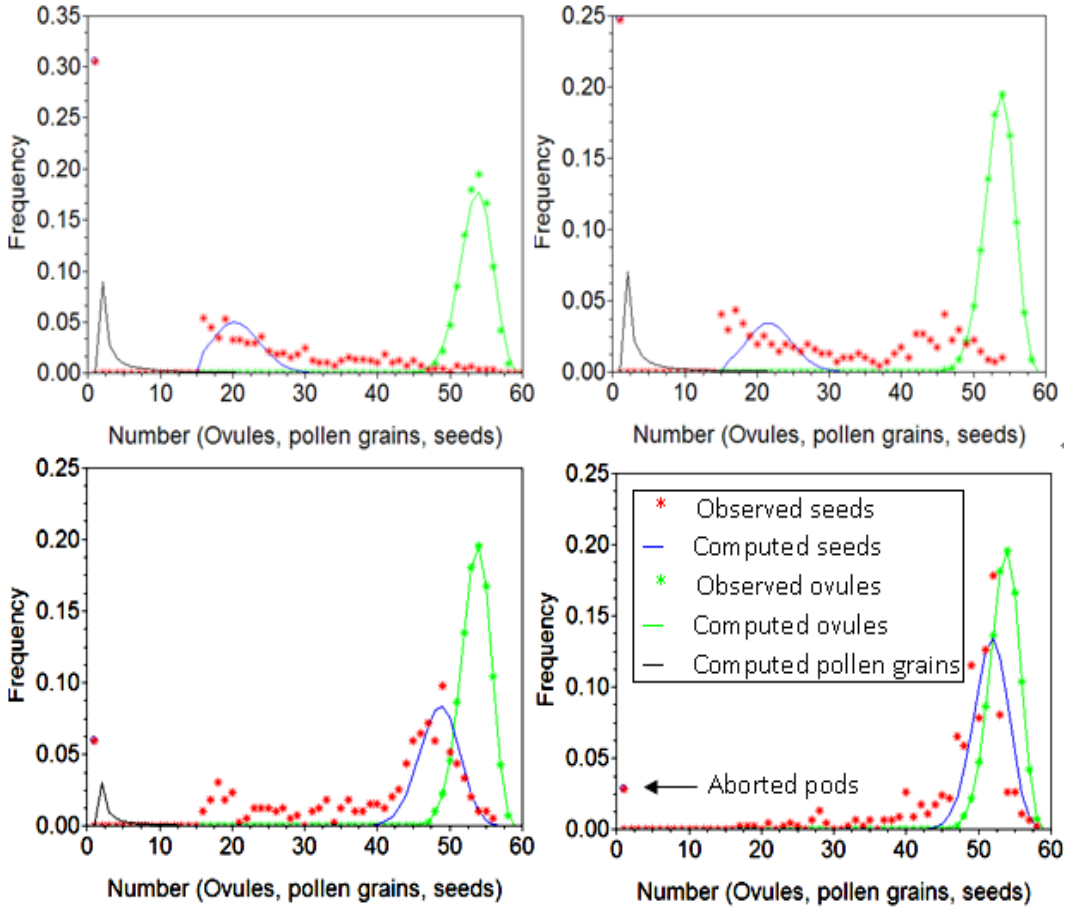


Figure 4.12: Estimations and measurements for the number of ovules and seeds per pod, the computed distribution of pollen grain number in cacao tree

Chapter 5

Conclusions and perspectives

5.1 Conclusions

The thesis investigated the variation of main yield components, including the number of ovules per pod, the number of seeds per pod and abortion rate of pods in WOSR. The factors influencing these yield components were analyzed, such as ovule fertilization, pollination limitation and competition for assimilates and architectural effects. Field experiments were conducted to investment the influence of these factors on yield components in WOSR. Continuous observations were carried out during the flowering period, and some treatments and measurements were performed to analyze the effect of different factors on seed production. In addition, the difference of the number of ovules and seeds per pod between varieties were investigated. Based on some previous studies in cacao tree, coffee tree and oil-palm, we developed a probabilistic model of flower fertility by combining some probability distributions in WOSR. The experimental data of different measurements and treatments were used to estimate the parameter values of the model, which can help us to distinguish the factors influencing the yield. Furthermore,rResampling method was used to estimated the stability of the model parameters. In the last, we used the model to simulate the flower fertility of other flowering plants. Accordingly, some main conclusions can be drawn:

1 The number of ovules per pod varied with pod ranks on the main stem (R0) and ramifications R1 and R4. The number of ovules per pod was small at the beginning of the stem, then remained constant along the inflorescence. But the number remained constant on the other ramifications R7, R9 and R11. Furthermore, the number of ovules per pod increased with the ramifications from top to bottom. The results indicated that plant architecure (pod rank and inflorescence position) has an effect on the number of ovules per pod. This difference could be due to the complex developmental patterns of inflorescences in WOSR.

2 The pod rank appeared to be the major determinant of the number of seeds per pod within one inflorescence. The number of seeds per pod remained constant

at the basal position and then decreased with the increase of pod ranks. In addition, the number of seeds per pod started to decrease later on the main stem (R0) than the ramifications, but the number of seeds per pod did not differ between ramifications (R1, R4, R7, R9 and R11). The decreasing pattern observed could be due to a limited access to assimilate because they have been depleted or intercepted by more proximal pods along the stem. In addition, the decrease of ovule viability could lead to the decrease of the number of seeds per pod.

3 The rate of pod abortion was large at the basal position, then remained constant and increased with the pod rank along the inflorescence. The number of aborted ovules behaved the same trend with the time of pod appearance. The results indicated that the number of pods and seeds depend on flowering time on the whole plant level.

4 The clipping treatments induced significant increases in the number of ovules, seeds and pods in the plants. When clipping the main stem or ramifications, the demand for assimilate and thus the trophic pressure in the entire plant decreases. Plants subjected to clipping treatments developed more pods and more seeds per pod than control plants that were not subjected to clippings. These results indicated that assimilate availability should be a cause of influencing the seed production.

5 The parameter of pollination varied with pod ranks, inflorescence positions and varieties. Thus, the variation of pollination could result in the variation of yield components. The parameter of seed viability increased and the probability for a pod to survive varied with clipping treatments. The results suggested that assimilate availability is the factor influencing seed production.

6 The CVs of model parameters were not large using resampling method (Jackknife and Bootstrap). The results demonstrated that the model parameters are stable and the estimations were reliable.

7 The model can simulate the distribution of the number of ovules and seeds per pod in soybean, and the estimations were good for cacao tree under good pollination condition. The results indicated that the model can be used to simulate the flower fertility of the other flowering plants. However, the model should be adjusted for each of flowering plant.

Taken together, our results indicate that in WOSR, the amount of available assimilates was the primary determinant of pod and seed production during the period of flowering and pod setting. The distribution of resources was significantly affected both by the position of a pod within inflorescences, and by the position of the inflorescences within a plant. Basally positioned pods had a distinct advantage in acquiring resources due

to their greater proximity and earlier development time. Increases in pod rank and ramification position affect appearing time, which can be observed through the change in assimilate availability on the entire plant. Furthermore, the estimated distribution parameter of pollen grain number indicated that pollination limitation could result in the variation of seed production. In addition, the decrease number of seeds per pod at the distal positions of the stem could due to ovule viability.

5.2 Innovation

1 **Application of modelling and statistics approaches in agronomy**
The biological processes were described by bio-mathematical method. Seed production depends on the successful completion of pollination and fertilization. In the thesis, winter oilseed rape flower fertility and processes of seed production were simulated with probabilistic distributions, by which the non observable or measurable parameters can be deduced such as the distribution of the number of pollen grains. Furthermore, the stability of model parameter was analyzed using the jackknife and bootstrap resampling methods.

2 **Description of temporal and spatial variability of yield components**
The impact of pod appearance time and pod position in WOSR on the variability of yield components was demonstrated by the measurements of the numbers of ovules per ovary, seeds per pod and pods per inflorescence. By combining experimental and modelling approaches, parameter estimations using the model were performed for the processes of flower fertility under different treatments and measurements to analyze the factors influencing the seed production.

5.3 Problem to be solved

- Because of the difficulty to measure the number of pollen grains in WOSR, we can not valid whether the distribution we chose in our model is proper. Some detailed investigates need to be made to observe the pollination conditions in WOSR.

- The number of seeds per pod and the estimation of the number of pollen grains on the ramification R11 was different from the other ramifications, we can not explain the reason. More studies on this phenomenon should be done.

5.4 Perspectives

This model is suited to simulate the processes of flower fertility in other flowering plants, which have synapomorphies in seed production. The thesis applied this model to the

soybean and cacao tree, the work were very preliminary.
Functional-Structural model Greenlab considers the inflorescences as an organ to simulate the plant development. The model can be linked to the Greenlab model to consider the flower development in detail.

Part III

Appendix

Appendix A

Formula for computing the probability of seed number

X, Y and Z, respectively, denote the numbers of pollen grains, ovules and fertilized ovules. We assume in the model that $Z = min(X, Y)$. Hence, we recall the equation [4.10]:

$$P(Z = k) = P(X = k)P(Y > k) + P(Y = k)P(X \geq k)$$

We use the theory of total probability to write the probability to get k fertilized ovules:

$$P(Z = k) = \sum_{y=0}^{N} P(Z = k/Y = y)P(Y = y)$$

$$P(Z = k) = \sum_{y=0}^{N} P(min(X, Y) = k|Y = y)P(Y = y)$$

$$P(min(X, Y) = k/Y = y) = \begin{cases} 0 & k > y \\ P(X = k) & k < y \\ P(X \geq k) & k = y \end{cases} \tag{A.1}$$

Hence we have:

$$P(Z = k) = P(Y = k)P(X \geq k) + \sum_{y=k+1}^{N} P(X = k)P(Y = y)$$

Lastlty, we assume that the number S of fertile ovules depends on Z with a Bernoulli process of parameter p, which means that:

$$P(S = i/Z = k) = C_k^i p^i (1 - p)^{k-i}$$

Likewise, we use the theory of total probability to compute the law of the random variable S:

$$\begin{aligned}
P(S=i) &= \sum_{k=i}^{N} P(S=i/Z=k)P(Z=k) \\
&= \sum_{k=i}^{N} C_k^i p^i (1-p)^{k-i} P(Z=k) \\
&= \sum_{k=i}^{N} C_k^i p^i (1-p)^{k-i} P(Y=k) P(X \geq k) \\
&+ \sum_{k=i}^{N} C_k^i p^i (1-p)^{k-i} \sum_{y=k+1}^{N} P(X=k) P(Y=y) \\
&= \sum_{y=0}^{N} C_y^i p^i (1-p)^{y-i} P(Y=y) P(X \geq y) \\
&+ \sum_{y=0}^{N} \sum_{k=i}^{y-1} C_k^i p^i (1-p)^{k-i} P(X=k) P(Y=y)
\end{aligned}$$

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Publications

1 **Wang, X.J.**, Mathieu, A., Cournède, P.H., Allirand, J.M., Jullien, A., de Reffye, P., Zhang, B.G. 2011. Variability and regulation of the number of ovules, seeds and pods according to assimilate availability in winter oilseed rape (*Brassica napus* L.). Field Crops Research, 122(1): 60-69.

2 **Wang, X.J.**, Mathieu, A., Cournède, P.H., Allirand, J.M., Jullien, A., de Reffye, P., Zhang, B.G. Stochastic Models in Floral Biology and its Application to the Study of Oilseed Rape (*Brassica napus* L.) Fertility. Third International Symposium of Plant Growth Modeling, Simulation, Visualization and Applications (PMA), Beijing, China, 2009. IEEE Xplore.

3 **Wang, X.J.**, Mathieu, A., Cournède, P.H., Allirand, J.M., Jullien, A., de Reffye, P., Zhang, B.G. Calibration of a probabilistic model of oilseed rape fertility to analyze the inter-variety variability in number of seeds. 6th International Workshop on Functional-Structural Plant Models (FSPM), California, USA, 2010.

4 **Wang, X.J.**, Mathieu, A., Cournède, P.H., Allirand, J.M., Jullien, A., de Reffye, P., Zhang, B.G. Effects of pod position and its appearance time on pod and seed abortion in winter oilseed rape (*Brassica napus* L.). 13th International Rapeseed Congress (IRC), Prague, Czech, 2011.

5 Zhang, B.G., Kang, M.Z., Letort, V., **Wang, X.J.**, de Reffye, P.. Comparison Between Empirical or Functional Sinks of Organs - Application on Tomato Plant. Third International Symposium of Plant Growth Modeling, Simulation, Visualization and Applications (PMA), Beijing, China, 2009. IEEE Xplore.